CHAPTER 26

Alphaviruses

AD-A218 296

Clarence J. Peters and Joel M. Dalrymple

INFECTIOUS AGENTS

The alphaviruses constitute an important genus of the *Togaviridae* family. They are transmitted by mosquitoes, and their major ecological maintenance strategy is passage from mosquito to vertebrate to mosquito. Thus, an understanding of their epidemiology requires an appreciation of the factors that regulate populations of arthropods, vectors, and their interactions, as well as knowledge of the viral genome and

C.J. Peters: Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701.

J.M. Dalrymple: Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701.

its phenotypic expression. In many cases, humans are not the major vertebrate amplifier, but rather an accidental target of virus infection with no significance in the further propagation of virus.

When humans are infected, the consequences can range from asymptomatic seroconversion to devastating illness (Table 1). The great majority of human infections with alphaviruses are subclinical or result in a transient and only temporarily incapacitating febrile illness. With some viruses, a small but important fraction of these infections will result in viral entry into the central nervous system and viral encephalitis. Others are characteristically associated with an acute arthropathy. At times the clinical description of diseases, the frequency of different disease manifestations, and the casual association of virus infection to low-incidence diseases may be ambiguous. This is a particular problem with alphaviruses because of the lack of med-

TABLE 1. *Alphaviruses and their association with disease*

Disease	Epidemics
Acute arthropathy	
Chikungunya (CHIK)	+
Mayaro (MAY)	+
O'nyong-nyong (ONN)	+
Igbo Ora	+
Ross River (RR) [*epidemic polyarthritis*]	+
Sindbis	+
Ockelbo [*Pogosta and Karelian fevers*]	+
Babanki	
Barmah Forest (BF)	
Systemic febrile illness (encephalitis)	
Venezuelan equine encephalitis (VEE)[a]	+
Everglades (EVE)	
Mucambo (MUC)	
Tonate (TON)	
Primarily encephalitis	
Eastern equine encephalitis (EEE)[a]	+
Western equine encephalitis (WEE)[a]	+
Highlands J (HJ)[b]	
No recognized human disease	
Aura (AURA)	
Bebaru (BEB)[c]	
Cabassou (CAB)	
Fort Morgan (FM)	
Getah (GET)[a]	
Kyzylagach (KYZ)	
Middelburg (MID)	
Ndumu (NDU)	
Pixuna (PIX)	
Sagiyama (SAG)	
Semliki Forest (SFV)[c]	
Una (UNA)	
Whataroa (WHA)	

[a] Also causes equine epizootics.
[b] Rare.
[c] Laboratory infection.

Morphology and Structure

Alphaviruses appear as 60–65-nm diameter particles that are essentially spherical. Both Sindbis (SIN) and Semliki Forest virus (SFV) have been studied extensively at the molecular level and serve as models of alphavirus architecture and replication strategy. The virion is composed of a nucleocapsid core containing RNA in intimate association with the nucleocapsid protein, which is surrounded by a lipid bilayer and an outer glycoprotein shell (169). Specific structural analyses of SIN and SFV show that the two glycoproteins (240 of each per particle) are arranged in an icosahedral surface lattice and contain hydrophobic sequences which span the bilayer (169,349). Glycoprotein spikes, consisting of the two envelope glycoproteins E1 and E2 in intimate association, constitute the external surface of the virion and participate in interactions with most cells and antibodies. The spikes appear as columnar trimers arranged on a T + 4 lattice in cryoelectron micrographs of vitrified particles (132). The lipid bilayer of the virus is polyhedral in appearance and is apparently derived completely from host-cell membrane during budding. Alphavirus cores appear as isometric particles of 40-nm diameter with a T + 3 arrangement. Glycoprotein spikes are arranged complementary to the nucleocapsid with 240 copies of each spike protein interacting with 180 copies of capsid protein (132,133,169,242). Reassembly of SIN virus cores *in vitro* demonstrates that viral RNA and nucleocapsid protein are all that is required for the self-assembly process (457). Budding at the modified plasma membrane of infected cells completes the assembly of mature virus particles.

Physical and Chemical Properties

The RNA genome of alphaviruses is a continuous single strand of positive polarity that is polyadenylated at the 3' end and capped with a 7-methylguanosine at the 5' end (419). All alphavirus genomic RNAs are of the same approximate size (sedimentation coefficients 42–49S); and with an increasing number of alphaviruses being cloned and sequenced in their entirety, precise length measurements are now possible (e.g., SIN genome is 11,703 nucleotides long) (418). Naked alphavirus genomic RNA is infectious, and full-length RNA transcripts of SIN cDNA clones have similarly given rise to infectious RNA (350). The ability to obtain infectious RNA transcripts from cDNA opens new vistas for experimentally determining the mechanisms of alphavirus virulence and the engineering of attenuated alphavirus vaccines by site-directed mutagenesis and other manipulations of the DNA clone.

The nonstructural proteins of alphaviruses are translated as a polyprotein from the 5' two-thirds of a genome-length messenger RNA (418). The specific functions of the four nonstructural proteins is not yet known for any of the alphaviruses. The structural pro-

ical infrastructure in many geographic areas where they are intensively transmitted to humans, as well as the inconvenience and cost of viral diagnostic procedures.

In this chapter we attempt to summarize basic concepts of the classification of these viruses, their ecological strategies and epidemic potential, clinical disease manifestations, and prospects for their control. Their replication strategy is discussed in detail in Chapter 25, but this chapter presents molecular data that bear on these biological issues. We concentrate on the viruses of greatest biomedical significance; but even in the case of very important viruses, many uncertainties exist in our knowledge base.

teins are translated from a 26S subgenomic messenger RNA as a polyprotein that is subsequently cleaved to form five polypeptides. This 26S mRNA originates from the 3' one-third of the genome. The 5'→3' gene order for SIN (418) is capsid protein (C), PE2, 6K, E1. PE2 is subsequently cleaved to form the envelope glycoprotein E2 as well as E3. The 6K peptide has not been reported as a structural component for any alphavirus (454) and the E1 envelope glycoprotein (418). Alphavirus proteins C, E1, and E2 are well-established structural components of all alphaviruses examined to date; and in addition, a structural E3 has been found associated with purified SFV (138). The role of these structural proteins as antigens and immunogens will be discussed throughout the chapter.

Molecular characterizations of a variety of alphaviruses show that genome organization and replication strategy are consistent features characteristic of the genus. Amino acid sequence homologies of 30–90% have been reported, with the nonstructural proteins showing greater conservation than the structural proteins (421). Comparison of the nsP4 (nonstructural protein 4) from SIN and Middelburg revealed 73% sequence homology (448 identical amino acids in perfect alignment). Interestingly, only 181 of these identical amino acids were encoded by the same codon, leaving 267 amino acids conserved but using alternate codons (421).

Conservation of protein structure apparently extends beyond the alphavirus genus in that SIN nsP4 shares homology with three plant viruses, each with a considerably different genomic organization: tobacco mosaic virus, alfalfa mosaic virus, and bromegrass mosaic virus (178). Similarities among alphaviruses and these particular plant viruses extend to the nsP1 and nsP2 proteins of SIN as well, and although the genome organization is quite different, the replication strategies of these viruses show remarkable similarities (6). It is tempting to speculate that the alphaviruses and the three groups of plant viruses diverged from a common ancestral protovirus (421). Since both arboviruses and many plant viruses replicate in insects, replication in arthropods may be a common element. The intimate association that many alphavirus vector mosquitoes have with plants throughout much of their life cycle certainly lends credence to the hypothesis of an evolutionary relationship.

Antigenic Composition and Determinants

All alphaviruses are related, with common antigenic determinants observed by serological cross-reactions in techniques such as fluorescent antibody (FA), enzyme-linked immunosorbent assay (ELISA), radioimmune assay (RIA), or complement fixation (CF). Clas-

sically, these assays employed mixtures of antigens such as infected cells or extracts of infected animal tissue and polyclonal antisera (37,47,76). Hemagglutination inhibition (HI) is another technique that, although cross-reactive throughout the alphavirus genus, nonetheless retains sufficient specificity to define six antigenic complexes or serotypes (Table 2). Identification of specific virus or subtype usually requires neutralization tests or modified HI tests. The kinetic HI test, for example, is extremely useful in differentiating subtypes of Venezuelan equine encephalitis (VEE) virus as well as geographic varieties of eastern equine encephalitis (EEE) and Ross River virus (RRV) (46,219,336,466,469).

The precise molecular species, subvirion component, antigenic determinant, or epitope participating in each of the serological procedures relating the alphaviruses have not been described. However, some of the more recent investigations employing extensive batteries of monoclonal antibodies have made significant progress in defining alphavirus antigens at the molecular level (358). Alphavirus nucleocapsids are antigenically quite similar and appear to contain both group-reactive and type-specific determinants (76,358). It has long been assumed that the abundant nucleocapsids found in infected cell culture and in infected animal tissue, such as suckling mouse brain, represent the predominant antigen detected in complement fixation and fluorescent antibody tests. More recent research has centered on the envelope glycoprotein antigens which bear receptors involved in hemagglutination as well as those necessary for initiating virus infection.

The E1 glycoprotein of SIN is responsible for the hemagglutinating properties of this virus (59,76,77), and comparable proteins probably provide this function for other members of the genus. Monoclonal antibodies to the E1 of SIN define five antigenic sites, but only one of these is normally exposed on the surface of intact virions (383,384,420). Although it was originally presumed on the basis of polyclonal antisera that only anti-E2 antibodies are capable of neutralizing virus infectivity, neutralizing monoclonal antibodies reacting with E1 have now been described for multiple alphaviruses (19,59,272,273,289,318,357,384,420). The other four sites not present on the virion surface are exposed on infected cell membranes, suggesting that SIN E1 undergoes a significant structural rearrangement during maturation (384). In addition to hemagglutination and neutralization, enhanced infectivity for cell culture and inhibition of red blood cell hemolysis have also been attributed to antibodies reacting with the E1 glycoprotein of SIN virus (21,59). Monoclonal antibodies to SFV have been used to describe six spatially distinct E1 epitopes using competitive binding assays. A similar analysis of the E1 glycoprotein of
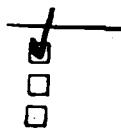
TABLE 2. Antigenic classification of alphaviruses[a]

Antigenic complex	Species (virus)	Subtype	Variety
Western equine encephalitis (WEE)	WEE Y 62-33 Highlands J (HJ) Fort Morgan (FM) Aura	Several	
	Sindbis (SIN)	Sindbis Babanki Whataroa Kyzylagach	Ockelbo
Venezuelan equine encephalitis (VEE)	VEE	I	A-B
		I	C
		I	D
		I	E
		I	F
		II Everglades (EVE)	
		III Mucambo (MUC)	MUC Tonate 71D-1252
		IV Pixuna (PIX) V Cabassou (CAB) VI AG80-663	
Eastern equine encephalitis (EEE)	EEE		North America South America
Semliki Forest	Semliki Forest (SFV)		
	Chikungunya (CHIK)	CHIK	Several
		ONN	Igbo Ora
	Getah (GET)	GET Sagiyama (SAG) Bebaru (BEB) Ross River (RRV)	
	Mayaro (MAY)	Mayaro Una	
Middelburg	Middelburg (MID)		
Nduma	Nduma (NDU)		
Barmah Forest	Barmah Forest		

[a] Adapted from refs. 37 and 39.

western equine encephalitis (WEE) virus has divided this antigenic molecule into eight epitopes, two of which are related to determinants also identified on VEE and SIN, and others which make up WEE complex-reactive and WEE virus-specific sites (191,358).

The alphavirus E2 glycoprotein (using SIN virus as the model) induces neutralizing antibody, and the majority of the polyclonal neutralizing antibody response to infection is directed at sites on this protein (77). Three separate antigenic sites capable of inducing neutralizing antibodies have been defined on E2 with the aid of monoclonal antibodies. Each of these neutralizing domains has been verified by the selection of monoclonal antibody escape mutants and the sequencing of point mutations responsible for altered antibody reactivity. Two of these three antigenic regions are apparently conserved among SIN strains, while the third appears to be strain specific (80,317,384,414,415). A neutralizing monoclonal antibody to SIN E2 has also been shown to activate virus particles that are nor-

mally not infectious by facilitating a more successful interaction between the virus and cells in culture (119).

Specific diagnostic antigens capable of readily differentiating each of the pathogenic alphaviruses do not yet exist; however, it is likely that linear epitopes defined by monoclonal antibodies could be synthesized and serve for serodiagnosis. As the battery of monoclonal antibodies to alphavirus antigens expands, better reagents for disease diagnosis and virus identification can be expected. Numerous virus-specific antibodies, as well as those with SIN strain and VEE subtype differentiating capabilities, are available, as are those reactive with the alphavirus genus, SIN, WEE, VEE, and EEE complexes (358).

Propagation and Assay in Cell Cultures

Alphaviruses produce an extensive cytopathic effect (CPE) in virtually all the common vertebrate cell cul-

tures examined. Primary cultures of avian embryo cells are often used as alphavirus-sensitive cultures capable of replicating virus to high titer; however, continuous cell lines of baby hamster kidney (BHK-21) and monkey kidney (Vero) are also employed extensively. Alphaviruses readily form plaques on most primary cultures or continuous cell lines, and the plaque assay serves as the most convenient procedure for quantitative estimates of infectious virus. The very first plaque assay of an animal virus was performed using the alphavirus WEE and primary chicken embryo fibroblast monolayers (96). Alphavirus plaque assays frequently approach a sensitivity of detection of a single infectious particle. Infectious virus can readily be recovered from the agar or agarose overlay medium in the vicinity of the plaque and plaque selection has been a useful procedure for the purification of genetically mixed virus populations. Variations in plaque morphology are easily recognized, and plaque selection, on the basis of size, has allowed numerous comparisons of different plaque variants.

The molecular biology of alphavirus infection of vertebrate cells has been studied in some detail. Certain steps, such as virus entry, are extremely well understood because SIN and SFV have been used as laboratory models to investigate the process (225). Alphavirus replication is the subject of Chapter 25, but some general comments regarding the conditions required to infect vertebrate cells may be relevant here as well. Virus attachment to cells is relatively slow with 30–40 min elapsed before binding 50% of the saturation density of the virus; yet once bound, the attachment is virtually irreversible. Virion binding appears to quantitatively increase with decreasing pH of the inoculum, and increased ionic strength can decrease alphavirus binding, particularly at pH values above 7 (268). Specific binding sites on the surface of most mammalian cells appear to be proteinaceous, or at least attachment can be inhibited by prior digestion with proteases. Attached virions are not evenly distributed on the surface of cells but exist as small patches and appear to concentrate near microvilli (130,409). After binding, alphavirus particles are endocytosed with a half-life approaching 10 min, and the endocytic pathway appears as the primary mechanism for entry (225). The early stages of alphavirus–cell interaction can be inhibited by a wide variety of weakly basic amines, suggesting a mechanism requiring reduction of endosome and lysosome pH (180). The initial release of progeny virions from infected cells can be observed within 3–4 hr at 34–37°C, and extensive cytopathology may be evident within 12 hr depending on the input multiplicity of the infecting virus and the particular cell line employed. Infectious virus release occurs via budding from the plasma membrane and can be inhibited by a variety of membrane-active compounds that either perturb normal membrane fluidity or inhibit virus cleavage of envelope glyprotein precursors such as the cleavage of PE2 to E2 and E3 (388).

In marked contrast to the cytolytic infection of vertebrate cells, alphavirus infection of most invertebrate cell lines results in a persistent infection without marked CPE. The absence of CPE is not absolute as was once believed. Cell lines derived from different mosquito genera, as well as different lines cloned from the cultured cells of a single species, have exhibited wide variation in both their ability to produce virus to high titer and their degree of cytopathology (32,376,439). Individual cell clones derived from a single parental culture vary considerably in their cytopathic response to alphavirus infection, and fusion of cells with "CPE plus" and "CPE minus" markers suggests that the CPE plus phenotype is dominant (376,439). By selecting a particular cell derivative of *Aedes albopictus* cells, adjusting the temperature of incubation, and varying the composition of the culture medium, SIN virus infection can vary between a cytolytic and a noncytopathic infection without appreciably affecting the yield of virus. Conditions leading to a cytopathic effect are also associated with a marked inhibition of cellular RNA and protein synthesis and the loss of the ability to induce heat shock proteins (429).

Although alphavirus infection of cultured cells initially involves virtually 100% of the cells, extended culture results in a decrease in virus RNA production and a reduction in the number of infected cells to as low as 2%. Such cultures remain refractory to superinfection by homologous virus. "Curing" of infected cultures can be accomplished by propagating cells in the presence of virus antiserum (32,193,351).

Invertebrate cell cultures are as sensitive as, or in some cases far more sensitive than, vertebrate cells for the detection of alphaviruses (e.g., 5,361). The relative ease of propagating established insect cell lines of known alphavirus sensitivity, coupled with their ability to grow in suspension without CO_2 buffers, as well as a temperature optimum at or near ambient in most temperate or tropical areas, makes these cultures ideal for alphavirus isolation and transport from field investigations.

Alphavirus replication pathways can be quite different in vertebrate and invertebrate cell cultures, but progeny virus RNAs and proteins are similar (417). Virus proteins produced in insect cells exhibit reduced carbohydrate and are devoid of sialic acid, because these cells contain no sialyl transferase activity (376). This has been reported to affect their interaction with the alternate C pathway and their clearance properties in the circulation (154). Major differences in virus in-

fection of insect cells include the strong suggestion that not all virus–cell interactions result in infection and high multiplicities of infecting virus frequently induce cell fusion. Electron microscopic examination of alphavirus morphogenesis in invertebrate cells shows virus assembly almost exclusively in cytoplasmic membrane-bound virus factories, and extracellular virus release may involve the exocytotic extrusion of virion-filled vesicles via fusion with the plasma membrane. A typical chronology of events leading to a persistent alphavirus infection of insect cells begins with infection of the majority of the cells in culture and high-titered virus release. This early infection may or may not involve cytopathology. Within days there is a significant decrease in the percentage of infected cells, and virus yields are also reduced. Over a period of weeks, the major virus population produces small plaques followed by the appearance of defective interfering virus particles and temperature-sensitive mutants. Virus titers continue to decrease with months and years in culture, and a slowly replicating low-titered alphavirus population is the only infectious product of long-term chronically infected cultures (reviewed in ref. 32).

Nature of Cell Injury

The precise mechanism by which alphaviruses produce CPE in any cell system remains unclear; however, numerous processes that could lead to cytolysis are involved in the virus replication process. Infection of cultured vertebrate cells is associated with a shutdown of host-cell RNA and protein synthesis, which ultimately contributes to cell death. A rapid inhibition of cellular DNA synthesis following infection with WEE virus has been attributed to an inhibitory factor with a direct effect on cellular DNA polymerase activity (possibly a phosphohydrolytic enzyme of precursor dNTPs) (233,234).

Increases in intracellular Na^+ concentrations following infection with SIN virus may also contribute to a selection for virus replication at the expense of host-cell functions. Virus protein insertion into the plasma membrane during alphavirus morphogenesis may adversely affect the ion-pump and/or osmotic integrity resulting in ionic imbalance. One hypothesis suggests that virus messenger RNA is preferentially translated at the elevated Na^+ concentration, which is inhibitory to host-cell messenger species (139,442,443). The appearance of SIN virus-induced cell leakiness, as measured by chromium release, coincides with both virus replication and a relative increase in saturated 18 carbon fatty acids, suggesting that a physical change in the lipids of the cell membrane may also contribute to CPE (315).

The absence of obvious cell injury or CPE, following alphavirus infection of certain insect cell lines, cannot be totally explained; however, the compartmentalization of virus replication in these cells, without significant modification of the plasma membrane, may help to maintain cellular integrity. Similarly, the physiology of the invertebrate cell simply may be better able to withstand the biochemical demands of alphavirus replication. It is of some interest that vertebrate cells virtually always exhibit CPE and cell death following alphavirus infection, with obvious parallels to the acutely ill vertebrate host. In contrast, cells from invertebrate sources generally replicate virus without cell injury similar to the noncytolytic infection of the mosquito vector, which remains infected for life.

Infection in Experimental Animals, Host Range

Alphaviruses exhibit a wide host range with a large number of different animal and arthropod species capable of being infected either experimentally or in nature. The vertebrate response to infection is extremely varied, ranging from inapparent infection (with or without detectable viremia) to severe encephalitis and death. Experimental infections have often been used to evaluate the potential role of a vertebrate host in the life cycle of a virus; in this setting low-passage field strains of the virus are used and the focus of interest is on the magnitude and duration of viremia and the serological response. The viremia data allow inferences about the likelihood of participation of the vertebrate in arthropod infection, and knowledge of the serological response can be invaluable in interpretation of field-collected epidemiological samples. In most cases the natural vertebrate host suffers no overt disease following infection. The principal vertebrate hosts for alphaviruses are birds, rodents, and primates, although wallabies, equines, bats, and other animals may play roles as well. In addition to the numerous wild rodents and migratory bird species found to be susceptible to alphavirus infection, poikilothermic reptiles and amphibians have been experimentally examined and found susceptible to infection with one or more alphaviruses (54).

Experimental infections have also been used to evaluate the pathogenesis of alphaviruses and the role of viral or host genetics in disease outcome (reviewed in ref. 154). There is no realistic animal model for the alphavirus syndromes characterized by fever, malaise, myalgia, arthritis, or rash, in part due to the difficulty of measuring many of these parameters in laboratory hosts. However, there are several systems to examine alphavirus-induced encephalitis. Horses develop fatal central nervous system (CNS) disease after WEE, EEE, and VEE infections, and this has been studied

for its intrinsic interest as well as its potential relevance to human disease. Several rodents have provided excellent models also, but the young mouse has been the most useful, developing fatal encephalitis after intracerebral (i.c.) inoculation of virtually all alphaviruses.

A variety of host factors affect the susceptibility of animals to experimental infection with alphaviruses, to include genetic background and age. Younger animals are uniformly more susceptible and resistance increases with age. Infant mice and freshly hatched chicks are highly susceptible in that these animals exhibit symptoms and frequently die following infection with several of the alphaviruses. Most alphaviruses are encephalitogenic in newborn mice but may require multiple brain-to-brain blind passages to bring neurovirulence to full expression in weanling or adult mice. Older animals are usually resistant to alphavirus infection by peripheral routes, although some remain susceptible to the encephalitis viruses administered i.c. Genetic variation probably plays an important role in alphavirus susceptibility, since different inbred strains of mice exhibit differential susceptibility to infection and disease.

Alphavirus strains, variants, and isolates have been described which differ markedly in their virulence for laboratory animals, particularly mice. Alphavirus strains of increased mouse neurovirulence have been selected by repeated i.c. passage of brain material from infected suckling mice and/or alternate passages into weanling or older animals (255). In contrast, alphaviruses with high passage levels in cell culture (18,250), temperature-sensitive mutants (14), small plaque variants (56), and viruses selected for rapid penetration of cells (209,317,366) have all been linked to reduced mouse virulence. Mouse virulent and avirulent virus pairs exist for several alphaviruses and offer abundant opportunity for in-depth analysis of virulence factors (26,209,317,366). The identification of specific mutations affecting virulence may have significant practical application for the development of live-attenuated alphavirus vaccines, but it remains to be established if the same mutations generating mouse avirulent viruses also dictate reduced virulence in other animal models and humans.

All alphaviruses pathogenic for humans replicate in and are transmitted by mosquitoes, and the infection of vector mosquitoes is generally thought to occur without significant pathology to the insect (82). Cytopathologic evidence of SFV infection of Ae. aegypti salivary glands has been described (292), although this mosquito is not a normal vector of SFV. Alphavirus infection of vector mosquitoes has not been associated with deleterious side effects or reduced survival rates; however, EEE has been shown to produce lesions in the midgut of the vector mosquito Culiseta melanura (450). The possibility that mosquito pathology is pre-requisite for alphavirus dissemination in the arthropod vector has implications for a complete understanding of virus transmission, epidemiology, and evolution.

PATHOGENESIS AND PATHOLOGY

Pathogenesis in Humans

The limited direct information available on the pathogenesis of alphaviruses in humans is summarized in sections pertaining to each virus. In general, infections are of short incubation and associated with a significant plasma viremia of a few days duration. During this period, fever, headache, and myalgia are common complaints and leukopenia occurs. These effects dominate the clinical picture of VEE and are prominent in chikungunya (CHIK), Mayaro (MAY), and sometimes EEE or WEE. Some of these effects are probably a consequence of circulating interferon and other soluble mediators, but direct muscle infection is common in mouse models and mildly elevated AST levels have been reported in humans. With some of the viruses (CHIK, RRV, MAY, SIN), a true arthritis occurs, and this is probably attributable to direct viral invasion. The rash that typically accompanies (RRV) or follows (CHIK, MAY) the arthritis may be due directly to viral replication and/or the immune mechanisms eliminating virus from the skin. With other alphaviruses a minority of infected humans (EEE, WEE, and rarely VEE) will suffer CNS viral invasion and develop encephalitis. The mechanisms of invasion are unknown, but direct viral damage is largely responsible for the clinical manifestations.

Termination of viremia is contemporaneous with the appearance of serum neutralizing or HI antibody whenever these events have been studied in humans. This and the supporting data from animal models lead to the conclusion that antibody is the dominant natural recovery mechanism from viremia, but this is only superficial insight into what may be occurring locally in nervous, integumentary, or musculoskeletal systems.

Experimental Animals

Current concepts of alphavirus pathogenesis have been derived from studies of experimental animals, primarily in the mouse and primarily focused on encephalitis (154). Several organ- or tissue-specific themes run throughout alphavirus pathogenesis, although infection patterns in humans often differ from those in rodent or other hosts. The local lymph node is presumed to be the site of primary replication in alphavirus infections, but the reticuloendothelial system becomes a major target organ in epizootic VEE (subtype IABC) infections.

In mice many of these viruses replicate extensively in muscle tissue, even producing a fatal myositis or myocarditis. This may be related to the pathogenesis of the myalgia and periarticular symptoms in humans, but there is no recognized nonhuman model for the arthritis seen after CHIK and other infections. Skin involvement has been seen in O'nyong-nyong (ONN)-infected mice or GET-infected horses, but the relationship to the rashes seen in humans is unknown.

The brain is, of course, a major target organ in rodents, horses, and humans, where a necrotizing encephalitis with neuronal destruction is the commonest acute lesion. In animals, acute (RRV) and late (SFV, VEE) demyelinization have been seen, as well as hydrocephalus secondary to ependymal scarring (RRV).

Liver, lung, kidney, and other major organs are largely spared in most alphavirus infections. The placenta and fetus are major target organs. In humans, WEE fetal infection is documented, with RRV and VEE suspected. In rodents, RRV, VEE, and SFV are known to infect the placenta and/or the fetus.

Immunology

Several generalities about alphavirus immunology can be addressed here and specifics discussed in the section appropriate to each virus. It should be noted that much of the work in this area has been done with selected variants of SIN or SFV and with inbred murine hosts, often resulting in a system that can be balanced to demonstrate the potential relevance of a given mechanism without necessarily addressing its more general importance.

Alphaviruses are readily neutralized by antibody (epitope requirements discussed under Vaccines). They also induce luxuriant cell-surface antigen early in infection, providing an excellent target to abort viral production; yet, since the virus–cell interaction is lytic, there is no requirement to eradicate infected cells *per se*. In fact, convalescent serum will prevent or cure systemic alphavirus infections, although if the CNS is involved, specific epitopes may be critical and not represented adequately in polyclonal sera (154). Protective epitopes can be expressed on virion and cell, or only on infected cells. Accessory cells are usually required for optimal protection, either through antibody-dependent cytotoxicity or clearance of opsonized virus.

The role of T-cell effectors in immunity has not been established. Cytotoxic T lymphocytes can only be demonstrated in some virus–mouse combinations and are not required for recovery, even of CNS infections (153,185,186). Delayed-type hypersensitivity can be demonstrated but, once again, its role in recovery or protection has been overshadowed by that of antibody.

Immunopathological events have not been an important theme in alphavirus pathogenesis, although rash and joint disease require further study. Although antibody enhancement of infection of Fc receptor-bearing cells has been demonstrated *in vitro*, there is as yet no suggestion that it plays a significant *in vivo* role.

Host factors unrelated to antigen-specific immune responses are important in alphavirus systems. In some virus–host combinations, modest immunosuppression has no deleterious effect on outcome. Young hosts are typically more susceptible to these viruses, and this may be reflected in susceptibility of primary cell cultures as well. Alphaviruses are highly sensitive to the antiviral effects of interferon, and in some systems the sensitivity of different viruses or the ability of different cells to achieve an antiviral state correlate with differences in outcome of infection.

Arthropods

An ecologically successful alphavirus must escape from the mosquito gastrointestinal tract after ingestion of a viremic blood meal and be secreted in the mosquito's saliva by the time the next blood meal is sought. The hurdles that the virus must overcome and the barriers to its entry into arthropod reproductive tissues form another pathogenetic sequence, although not complicated by a virus-specific immune response (168).

GENETICS

Theories concerning the evolution of alphaviruses have been stimulated by the recent comparison of numerous member viruses at the nucleotide sequence level. Speculation that all positive-sense RNA viruses evolved from a common ancestor has received support from studies demonstrating sequence similarities in otherwise diverse viruses infecting plants, animals, and insects (422). Logical extension of the hypothesis describing a common ancestral protovirus requires the evolution of alphaviruses via the recognized mechanisms of genetic change, mutation, selection, and possibly recombination. A very high rate of mutation for RNA viruses has been estimated on the order of 10^{-4} per nucleotide per replication cycle. Antigenic variants of SIN virus have been detected at frequencies between $10^{-3.5}$ and 10^{-5}, while other estimates suggest mutation rates approaching 10^{-7} (97,416,422). Selection against deleterious mutations would be obvious, and the selective pressure of antibodies in an otherwise susceptible population of vertebrate hosts has been a significant factor in the evolution of other RNA viruses

and presumably plays some role in the natural antigenic diversity of alphaviruses.

Recombination has never been reported in laboratory experiments with alphaviruses; however, sequence analysis suggests the alphavirus WEE actually arose by recombination between EEE virus and SIN or a very closely related SIN-like virus (159,422). Although it is difficult to imagine the overlapping habitats of an Old World and a New World virus that would allow a proximity prerequisite for recombination, the sequence data indicate that the 5' region of the WEE virus genome, to include the nonstructural proteins and the nucleocapsid protein, is derived from the EEE virus parent, while the 3' terminal 80 nucleotides and the genes for the envelope glycoproteins are more closely related to the Old World SIN virus. Hence, the origin of WEE virus by recombination yields a virus with encephalitogenic potential more like EEE but with antigenic properties similar to SIN virus. Genetic shift, drift, and recombinational elements will most likely be documented with increased frequency as more alphaviruses are sequenced.

Genetic factors contributing to alphavirus virulence and pathology in laboratory animals have been the subject of intense investigation motivated by the derivation of avirulent strains for use as human vaccines. A simple concept of virulence involves rapid replication in the host with the development of pathologic lesions prior to the initiation of a protective immune response. Such early hypotheses explained the increase in mouse neurovirulence associated with frequent mouse passages that appeared to overcome the host's immune response and correlate with overall growth rates, total virus yields, and onset of encephalitic signs after inoculation of relatively small doses (118,382). SIN variants of increased weanling mouse neurovirulence have been selected from the relatively avirulent AR339 parent by alternate brain-to-brain passage in suckling and weanling mice (153). In contrast, a collection of SFV variants derived from wild-type virus has exhibited marked differences in relative virulence in rabbits, guinea pigs, and mice; yet all variants exhibit comparable replication efficiencies and are equally lethal for suckling mice (26).

Molecular characterization of virulent–avirulent alphavirus pairs has recently attempted to relate such phenotypic markers to virus gene function. Sequence comparisons of the virulent Trinidad strain of VEE and a derivative vaccine, TC-83, obtained following 83 *in vitro* passages in guinea pig heart cells, revealed six amino acid differences in the structural proteins of these two viruses. Five of these changes were nonconservative changes in E2, while the sixth was a conservative change in E1, suggesting a role of the E2 glycoprotein in virulent phenotypes (18,206,288).

A single amino acid substitution in the E2 protein of

SIN virus (arginine for serine at E2 position 114) has been shown responsible for reduced neurovirulence for neonatal mice (79). In addition to attenuation in mice, this single sequence change results in accelerated penetration of BHK cells and variation in a specific epitope on E2 recognized by virus-neutralizing monoclonal antibodies. These studies define a specific domain on SIN E2 that plays a key structural role in maintaining the multiple biological functions of the SIN envelope. Sequence differences observed between mouse-avirulent and mouse-virulent strains of RRV identify two differences in E2, one of which is at residue 119, which is close and possibly related to position 114 of SIN (107). VEE variants selected for accelerated penetration in cell culture coselected for mutants that were attenuated for mice. Although not all mouse avirulent mutants were equally attenuated in hamsters, these studies demonstrate that the genetic link between rapid penetration and mouse attenuation is not restricted to SIN virus (209).

All alphavirus virulence cannot be explained simply on the basis of the integrity of a single "virulence-specific" locus. Antigenic variants selected by their resistance to critical neutralizing monoclonal antibodies retain the arginine for serine substitution at E2 position 114 but have lost rapid penetration properties and have resumed a virulent phenotype. Such reversion to virulence can be explained by an additional mutation in E2 which apparently acts as a suppressor mutation (79,317,318). Additional mutants from a more virulent South African strain of SIN virus were obtained by selecting rapid penetration variants in BHK cells and shown to be attenuated in neonatal mice. Once again, one class of attenuated mutants contained the arginine for serine substitution at E2 position 114, but another class of attenuated mutants contained only a single point mutation at the first amino acid of E2. A substitution of asparagine for serine in this first position generates a new site for N-linked glycosylation, which appears to be utilized and apparently blocks cleavage of PE2. Mutant virions of the attenuated phenotype contain the uncleaved precursor PE2 rather than the mature E2 glycoprotein (366).

The influence of selected SIN gene regions on mouse neurovirulence has been examined using infectious RNA transcripts from SIN virus cDNA clones (350). The first infectious SIN viruses derived from cDNA of high-passage, heat-resistant laboratory strains were attenuated for suckling mice. Replacement of the E1 glycoprotein and 6K genes with comparable genes cloned from a virulent SIN strain gave rise to virulent virus. Only three amino acid changes in the E1-6K genes differed between the two viruses. E2 changes can obviously influence mouse neurovirulence as well as those reported for E1. Substituting an arginine for serine at E2 residue 114 in the virus made virulent by

E1-6K gene substitutions again resulted in an attenuated SIN virus (335). Additional recombinant viruses generated via substitutions into an infectious SIN clone delineate a number of changes in E2 and E1 that can lead to decreased virulence, suggesting that virulence is truly polygenic and defines an actual gradient of neurovirulence (255). Continued description of genome alterations affecting alphavirus virulence will undoubtedly lead to a precise definition of gene loci involved in virulence attenuation. The development of candidate vaccine strains using infectious RNA transcripts of alphavirus cDNAs that have been specifically engineered for attenuation would appear as a reasonable approach to future human vaccines.

PROSPECTS FOR CONTROL

Ecology

We refer to the two basic ecological strategies of these viruses as "A" and "B." In A cycles, the major amplifier is a nonhuman vertebrate and the arthropod host is a mosquito that feeds regularly on that vertebrate. In B cycles, the major viremic amplifier is human and the arthropod host is *Ae. aegypti* or another mosquito with a close relationship to humans. Typical A cycles might include WEE, birds, *Culex tarsalis*; CHIK, monkeys, *Aedes africanus*; EEE, birds, *Culiseta melanura*; RRV, marsupials, *Ae. vigilax*. Examples of B cycles are CHIK, humans, *Ae. aegypti*; or RRV, humans, *Ae. polynesiensis*.

In typical type-A cycles, humans can be protected by vaccination of the amplifier host. This is feasible, and perhaps even essential, for epizootic VEE and may also have limited utility in EEE pheasant epidemics, but it is not generally applicable. Deforestation and other consequences of the brutalization of our planet may limit the habitat of some amplifiers (e.g., monkeys) and thereby secondarily diminish the opportunities for virus circulation.

Control of mosquitoes important for type-A cycles can be effective but may be quite difficult. Simple approaches such as empiric applications of insecticides to large areas are of limited effectiveness, particularly because of expense, mosquito resistance, and environmental concerns. Typical breeding sites are treeholes (*Aedes* vectors of CHIK), marshes (*Aedes vigilax*–RRV; *Cx. annulirostris*–RRV), shaded freshwater swamps (*Culiseta melanura*–EEE), shaded tropical forest (*Culex [Melanoconion]*–enzootic VEE), and seasonally flooded irrigated areas (*Cx. tarsalis*–WEE). Drainage of selected swampy areas or flood plains in contiguity with human settlements may be feasible in some cases, but in today's world, destruction of mosquito habitats is less and less often a prac-

tical solution. Certain mosquito species (*Cx. tarsalis*–WEE, *Cx. tritaeniorhynchus*–GET) are particularly adapted to irrigated agricultural areas, and their control requires an ecologically perceptive, epidemiologically insightful approach utilizing surveillance to trigger rational programs to reduce mosquito larval and adult populations below threshold levels for disease transmission (103).

Control of mosquitoes crucial to type-B (human amplifier) cycles usually involves control of *Ae. aegypti*. This is an attainable goal conceptually.

The mosquito breeds in clean, still water sources such as drinking water containers, discarded plastic vessels, or old tires. Programs based on breeding site destruction were developed to deal with *Ae. aegypti*-transmitted yellow fever by Gorgas in Cuba and subsequently perfected in Panama. Application of the same principles resulted in elimination of this vector from the southern United States and other areas in the Americas, but after relaxation of this expensive, labor-intensive approach, reinfestation of much of the region has occurred. Massive community efforts have aborted *Ae. aegypti*-borne viral epidemics, but considerable obstacles to prolonged and effective control exist: lack of piped water leading to extensive use of water storage jars in homes, cultural practices, and community attitudes (331). While elimination of larval breeding sites is a proven approach to *Ae. aegypti* control, success depends on appropriate water supplies and an educated community willing to assume responsibility for control of breeding sites. Insecticides have also been of limited utility in killing adult *Ae. aegypti* (121,344). Control of other peridomestic vectors such as *Ae. albopictus* (173) or *Ae. polynesiensis* (222) present similar problems, in addition to the possibility of their persistence in alternate breeding sites.

Vaccines

Although there are no alphavirus vaccines applicable for widespread use, the success of several immunogens in protecting laboratory workers predicts a promising future for vaccines. Human vaccines against EEE, WEE, and VEE are available under the usual procedures applicable to investigational new drugs (IND). A new live-attenuated CHIK vaccine has been shown to be safe in a limited number of volunteers and after field evaluation may prove to be broadly useful. The live-attenuated TC-83 VEE vaccine has not only provided direct protection to humans but was instrumental in containing the equine epizootic spread of VEE in Texas in 1971. Successful veterinary vaccines also ameliorate the economic impact of alphavirus disease (EEE, WEE, VEE, GET in horses, or EEE in pheasants).

The success of nonreplicating immunogens and the high efficiency of passive antibody in protection emphasize the importance of the humoral immune response in protection against alphavirus disease. Analysis of protective responses has obviously focused on critical domains on the alphavirus glycoprotein spike (153,383,420). Multiple epitopes on each of the E1 or the E2 glycoproteins are capable of inducing and interacting with protective neutralizing antibody and complement is apparently not required for protection (19,289,359,383,384,413,441). Nonneutralizing antibody is also capable of passive protection of experimental animals and may function by reacting, together with complement or accessory cells, on the surface of infected cells, resulting in early lysis or removal of cells and a significant decrease in infectious virus yield (58,272,383,384,420). Antigenic determinants responsible for such interactions are hidden or cryptic on the surface of the virion itself, but mild denaturation exposes these epitopes (191,384). An alphavirus group-reactive epitope that induces protective antibody in experimental animals is one of these conformationally dependent determinants that is only exposed on infected cell-surface membranes or denatured virions. The mechanism of such cross-protection is also not known, although antibody-mediated infected cell cytolysis is suspected to play a role. As a direct result of an ever-increasing battery of alphavirus monoclonal antibodies, alphavirus epitopes are being defined with greater precision and a current map of functionally defined antigenic determinants provides the background for development of engineered vaccines (441).

Cellular immunity, although not of established importance in alphavirus immunology, may provide another avenue for protection. Studies with cytotoxic T cells in mice suggest that this response is cross-reactive and confers cross protection among alphaviruses but depends on antigen denaturation, dose, and route of presentation. Levels of protection are often low and the responses short-lived (115,140,226,238,243,303, 325–327,411,464,465). Cytotoxic macrophages have also been implicated in the protective immune response (348). Although the vast majority of these studies measuring cellular responses emphasized the cross-reactivity of the T-cell response, other investigators observe virus-specific T-cell activity using lymphocyte proliferative responses or interleukin 2 production. These responses remain virus specific regardless of whether animals are hyperimmunized with a single virus or cross-immunized with different alphaviruses (441).

Recombinant vaccinia virus expressing VEE virus antigens shows considerable promise for engineered alphavirus vaccines of the future (227). Significant in the search for improved alphavirus vaccines is the recent ability to generate infectious RNA transcripts from cDNA; first with SIN virus (350), and more recently with the Trinidad donkey (IAB) vaccine strain of VEE (81). The transfer of specific gene elements into the infectious clone to evaluate the influence of the expressed product on either virus virulence or induction of an immune response greatly increases the probability of future alphavirus vaccine success (255,335). The potential for substituting multiple antigenic determinants from different alphaviruses into a single avirulent infectious clone and/or to maximize the expression of cross-protective alphavirus epitopes in engineered recombinant vaccine viruses are approaches to the control of alphavirus disease that are becoming real possibilities.

Chemotherapy

A number of promising antiviral compounds have been identified in cell culture and animal models (190,423), but none is near clinical trials. Ribavirin, a promising broad-spectrum antiviral, has been effective in therapy of hemorrhagic fevers caused by viruses of the family Bunyaviridae and Arenaviridae (330). Although it inhibits alphavirus replication in cell culture, its activity in alphavirus animal models has been disappointing and acute penetration into the central nervous system is poor.

ALPHAVIRUSES ASSOCIATED PRIMARILY WITH FEVER AND POLYARTHRITIS

Chikungunya Virus

Two or three centuries ago CHIK virus was probably an infection of primates in the forests and savannahs of Africa maintained by sylvatic *Aedes* mosquitoes, as it continues to be today. However, today CHIK is also responsible for extensive *Aedes aegypti*-transmitted urban disease in cities of Africa and major epidemics in Asia. The crippling arthralgia and frequent arthritis that accompany the fever and other systemic symptoms of CHIK infection are clinically distinctive, and their persistence during convalescence is an important factor in assessing the biomedical impact of this disease. Several other togaviruses of the alphavirus genus O'nyong-nyong (ONN), Igbo Ora, MAY, RRV, and some strains of SIN have been associated with a similar syndrome, and, of course, the togavirus rubella is also arthritogenic.

Using the prominent articular manifestations and other clinical criteria as a retrospective guide, it seems likely that CHIK caused epidemics in Indonesia (1779), East Africa (1823,1870), India (1824,1871,1901, 1923), the Far East (1901–1902), West Africa (1925), and even possibly the southern United States (1827–

1828) (42,245). However, the original isolation of the virus (364) and the modern description of the disease occurred during a 1952–1953 epidemic in Tanzania (254,353). It was also during that epidemic that the local tribal word "CHIK" (chikungunya) meaning "that which contorts or bends up" came to be applied to the virus and the disease. Identification of the virus has led to the understanding of its transmission between primates and *Aedes* mosquitoes of the subgenera *Stegomyia* and *Diceromyia* in endemic transmission in Africa; the importance of *Ae. aegypti* in urban and other epidemics; the massive impact of these epidemics on public health; and the extensive, regular infection of residents of and travelers to sub-Saharan Africa (Fig. 1). Finally, with the reinfestation of the Americas with *Ae. aegypti* and the introduction of *Ae. albopictus*, the possibility of epidemics in the Western Hemisphere must be considered.

Infectious Agent

CHIK resembles other alphaviruses in most properties. It is quite sensitive to "auto interference" (364,396) and, when passed in infant mice, must be diluted 10^{-2} to 10^{-3}. Not unexpectedly, several biological differences have emerged when geographically different strains at different passage levels are compared for their growth in cell culture or mosquitoes, but the overall significance of these differences is unclear (301,307,434). The antigenic comparison of a geographic library of CHIK strains utilizing both polyclonal and monoclonal antibodies revealed a conservation of major antigens associated with HI and neutralization. CHIK virus variants selected by cell culture passage were of lower virulence for mice; small plaque variants were also correlated with reduced virulence, while the Ross strain of CHIK virus maintained by over 176 passages in suckling mouse brains has shown increased mouse neurovirulence and large plaque morphology with conservation of antigenic properties (13,34,56,57).

Pathogenesis and Pathology

In infant mice CHIK causes fatal necrotizing encephalitis with an accompanying myocarditis and myositis (453). Some strains cause hemorrhagic disease in rodents (161). Both African and Asian monkeys develop high viremia without apparent illness (215,279). Birds are refractory to viremia and often do not even exhibit an antibody response.

In humans, CHIK virus typically produces disease about 48 hr after mosquito inoculation. Patients have high viremias, often exceeding 6.0 $\log_{10}$ SMICLD$_{50}$/ 0.02 ml during the first 2 days of illness (41,279,396).
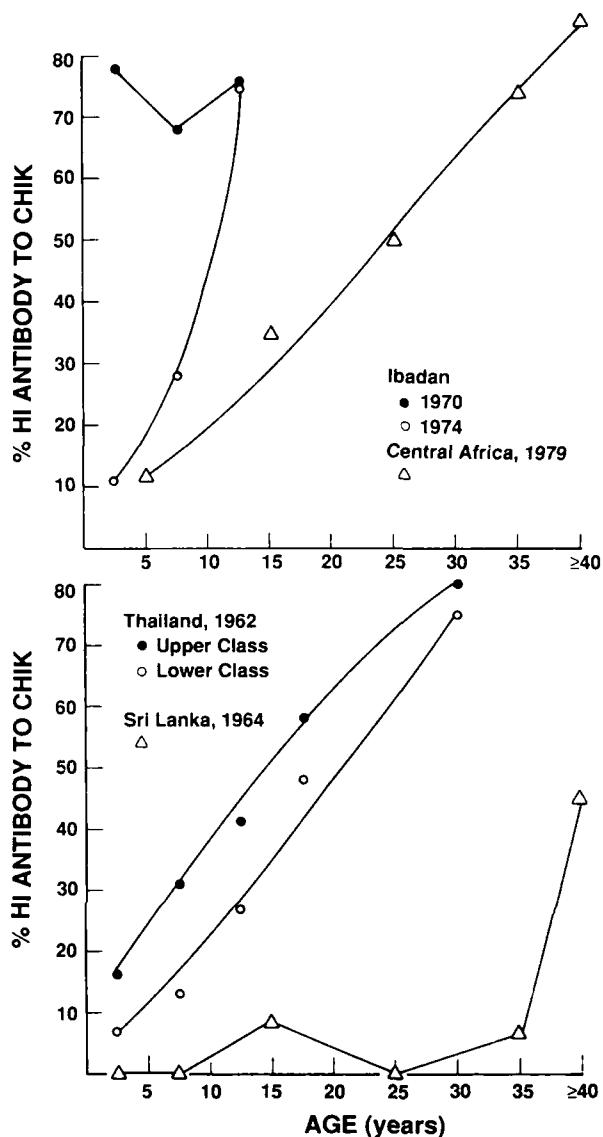

FIG. 1. Chikungunya infection rates determined by prevalence of HI antibodies in different populations. **Top:** An endemic pattern is seen in sera taken from residents of villages in the savannahs of the Central African Republic. Virus can be isolated from *Aedes africanus* and *Aedes opok* mosquitoes in nearby gallery forest most years. Contrast with Ibadan, Nigeria, where a major *Aedes aegypti*-transmitted epidemic occurred in 1969, leaving extensive immunity in even the youngest age groups. By 1974 susceptibles had accumulated in the youngest age groups and another epidemic occurred later that year. **Bottom:** Antibody surveys in Bangkok, Thailand, in 1962 suggested continuous urban transmission over several years, with somewhat higher risk in neighborhoods in the lower economic districts. Contrast with Colombo, Sri Lanka, where the last significant CHIK activity appears to have been more than 30 years before the survey sera were obtained. Data from Hermon (182), Saluzzo et al. (368), Halstead et al. (165), Tomori et al. (438), and Moore et al. (298).

Viremia, HI Antibody and Fever in Chikungunya
Infection, Vellore, 1964
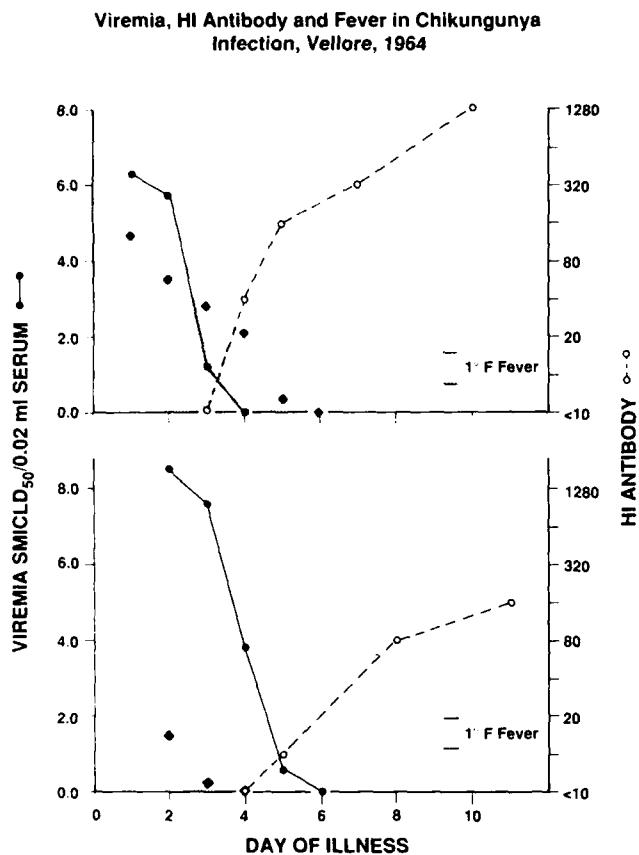
FIG. 2. Viremia, HI antibody, and fever in chikungunya infection, Vellore, South India, 1964 (41). These two infants illustrate the high but brief viremias typical of CHIK fever. Note appearance of HI antibody with cessation of viremia. Neutralizing antibody is detected in the same time frame as HI and CF antibody a few days later.

Viremia declines around day 3 or 4, usually disappearing by day 5. As viremia fades, HI and neutralizing antibody can usually be detected (Fig. 2). Thus, it seems plausible that serum antibody, which is active in mouse protection tests, is a major mechanism for recovery.

Although the clinical manifestations of CHIK infection seem to be less prominent in children, a small minority develop serious or even fatal disease. It is not clear whether the very rare fatalities (182,196) are specifically due to CHIK infection itself or perhaps to hyperpyrexia or exacerbation of an underlying convulsive disorder. In any case, CHIK infection of infants and young children can occasionally produc·' a moderately severe, nonfatal disease with mild hemorrhagic manifestations (196,224,313) and in adults can infrequently be associated with clinically insignificant hemostatic abnormalities such as cutaneous petechiae, abnormal tourniquet tests, or mild gingivorrhagia (84,85,141). These hemostatic abnormalities have an *in vitro* correlate; CHIK virus adsorbs to human plate-

lets and promotes clumping (61). This could signal an interaction with the potential to lead to an acquired platelet defect *in vivo*.

Very little is known of the pathology of CHIK infection in humans. Biopsy of a skin lesion showed lymphocytic perivascular cuffing and erythrocyte extravasation from superficial capillaries (120). In one patient with chronic arthropathy, an atrophic-appearing synovium was biopsied during arthroscopy but was histologically normal (29), and in an atypical case with destructive joint disease, open biopsy revealed pannus formation, synovitis with acute and chronic inflammation, and local immune response manifested by germinal center formation (31).

Clinical Features

CHIK is an acute viral infection of brusque onset, heralded by fever and severe arthralgia, followed by constitutional symptoms and rash, and lasting a brief period of 1–7 days (29,84,85,120,141,223,259,261,266, 278,353,354,370). The incubation period is usually 2–3 days, with a range of 1–12 days. Although descriptions of clinical manifestations have varied with different series, the most characteristic feature is abrupt transition from health to a state of incapacitating arthralgia and fever. Ongoing tasks can be interrupted and the exact hour of onset is frequently recalled by the patient. Fever rises abruptly, often reaching 39–40°C and accompanied by shaking chills. This acute phase typically lasts 2–3 days, with a range of 1–7 days. The temperature may remit for 1–2 days, resulting in a "saddle-back" fever curve.

The arthralgias are polyarticular, migratory, and predominantly affect the small joints of the hands, wrists, ankles, and feet, with lesser involvement of larger joints. Sites of previous injury are often selectively involved. Severely afflicted patients characteristically lie still, in an attitude of flexion, and complain bitterly of pain when forced to move. Pain on movement is worse in the morning, improved by mild exercise, and exacerbated by strenuous exercise. Swelling may occur, but fluid accumulation is uncommon. Patients with milder articular manifestations are usually symptom free within 1 to a few weeks, but more severe cases require months to resolve entirely, and in rare patients, signs and symptoms of articular disease persist indefinitely. Generalized myalgia, as well as back and shoulder pain, is common.

Cutaneous manifestations are typical. Many patients will present with a flush over the face and neck. This is usually followed by a rash generally described as maculopapular, but in some series identified as macular. This rash may occur as early as the first day or as late as the tenth day of illness, but typically makes

its appearance on days 2–5, often contemporaneous with defervescence. It lasts 1–5 days and its recurrence, sometimes with recrudescence of fever, is described. The trunk and limbs are commonly involved, but face, palms, and soles may also show lesions. Pruritis or irritation may accompany the eruption. The rash may simply fade or it may desquamate.

Petechiae may occur alone or in association with the rash. Bleeding from gums is not rare, but no significant hemorrhagic manifestations occur. A positive tourniquet test is found in a small proportion of patients.

During the acute disease, most patients will have headache, but it is not usually severe and does not dominate the clinical picture. Photophobia and retroorbital pain also occur with frequency but are not usually severe. Conjunctival infection is prominent. Some patients will complain of sore throat and have pharyngitis on examination. The majority of patients will be anorectic or nauseous, many will vomit, and some will have abdominal pain or tenderness. Adenopathy occurs but is not florid, nor is tenderness prominent. Other findings of less-established significance include ankle swelling during recovery, dry cough, oral ulcers, burning oral sensations, and swollen, reddened pinnae.

There is no systematic data on case/infection ratios, but the majority of adult infections are probably symptomatic. For example, laboratory staff infected during a CHIK epidemic experienced typical disease, and only 1 staff member later found to be seropositive was asymptomatic (41). Furthermore, most recognized cases (60–80%) have rash and joint complaints. Mild and atypical cases are probably much more common in children.

Clinical laboratory findings are not outstanding. A significant minority of patients will have leukopenia with relative lymphocytosis, but most will have a normal blood count. The platelet count may be modestly decreased, but rarely to significant levels. A proportion of the few AST determinations performed have been modestly elevated. The erythrocyte sedimentation rate is significantly elevated (85), and the C-reactive protein is positive (223) in acute disease.

The long duration of joint symptoms results in a major impact on active adults in rural societies. The potential for permanent arthritic sequelae also exists and requires further definition. For example, in one group of 20 patients identified during an outbreak, examination by a rheumatologist 4 months after acute disease showed residual tenosynovitis and periarticular soft tissue swelling in eight. Three subjects still complained of severe symptoms, and more than half complained of morning stiffness or had decreased grip strength. The three most symptomatic patients had positive C-reactive protein tests, and two had low-titered rheumatoid factor (223). In a retrospective study of 107 cases, only one-third had full recovery of joint

function within a few weeks, although 88% eventually were symptom free. About 5%, predominantly older patients, had residual stiffness and/or discomfort of questionable causation and modest significance. However, an additional 5% had persistent joint pain, stiffness, and recurrent effusions 3–5 years after infection. In these patients, erythrocyte sedimentation rates were slightly elevated, serum rheumatoid factors were negative, and CHIK HI antibody titers tended to be somewhat higher than expected (1:160 to 1:640) (29). None of the patients with chronic involvement had erosive joint changes on X-ray. A single case report described a man with a compatible clinical history of acute disease during a documented CHIK epidemic who developed destructive arthritis active for a period of 15 years and who was found to have serological evidence of CHIK infection (31). Interestingly, four of five patients with chronic arthropathy following CHIK infection were found to be HLA B27 positive (120).

CHIK infection has a somewhat different picture in younger patients (163,196,259,261,298,313). Arthralgia and arthritis occur but are less prominent and last a shorter period of time. Rash may be less frequent in pediatric cases; but in infants and younger children, prominent flushing and early appearance of maculopapular or urticarial eruption may be a useful indicator of CHIK infection.

In Asia, several virus isolations have been made fron severely ill children diagnosed as having hemorrhagic fever (162,167,182,196,224,396). Under the circumstances, it was difficult to define the relative roles of hyperpyrexia, convulsive disorders, simultaneous circulation of dengue viruses causing hemorrhagic fever, and other intercurrent diseases.

Perhaps the most relevant data on the true spectrum of pediatric CHIK infection comes from longitudinal virological studies in Bangkok, Thailand (163,313). Among 17 outpatients (5.6% of clinic visits), one-third presented with vomiting, 71% had pharyngitis on examination, and 24% were described with facial flushing, but none was noted to have arthralgia, arthritis, or rash. When compared to DEN and other patients, they presented earlier in their illness (within 24–48 hr) with higher temperatures (two-thirds were 39°C or higher and one-fourth exceeded 40°C). Hospital-based studies concentrated on patients with suspected hemorrhagic fever. About 8% of these children proved to have CHIK infection, one-tenth the number with DEN virus infection. CHIK-infected patients presented with high fever (69% were 39°C or above), headache (68%), injected pharynx (90%), vomiting (59%), abdominal pain (32%), constipation (40%), diarrhea (16%), cough (23%), and adenopathy (31%), all features that resembled DEN hemorrhagic fever cases. CHIK patients frequently manifested maculopapular rash (59%), conjunctival injection (56%), and myalgia or arthralgia

(40%), findings that were significantly less often seen in DEN patients. As in DEN, tourniquet tests were often positive (77%) and scattered petechiae (31%) or epistaxis occurred (12%), but in contrast gastrointestinal bleeding or shock were not observed, and most patients defervesced within 4–5 days. Thus, milder cases resembling the viral hemorrhagic fever syndrome could be attributed to CHIK virus, but DEN virus was more often responsible and was the sole cause of severe and fatal infections.

A number of children with convulsions during acute CHIK infection have been reported. The rapid rise in temperature to 39 or 40°C that characterizes the onset of CHIK infection in the younger age group is likely to result in a high incidence of benign febrile convulsions; however, the occasional occurrence of focal seizures, repeated seizures, and convulsions in older age groups suggests that direct brain involvement can occur. Patients with CHIK infection and suggestive meningeal or encephalitic signs have also been reported (274,313). The possibility that CHIK virus causes central nervous system disease in children remains open, but the incidence must be low and the severity only modest.

Myocarditis has been suggested as a sequela of CHIK infection (263,316), but available data are not adequate to confirm or deny the association.

Diagnosis

The definitive diagnosis can only be made by laboratory means, but CHIK should be suspected in any febrile patient resident in, or returning from, sub-Saharan Africa or in temperate and tropical regions of Asia as far east as Weber's line (vide infra). Most arbovirus infections, including CHIK, present a particular risk to rural travelers; however, CHIK is also widely transmitted in urban areas because of its Ae. aegypti association. An individual case may be indistinguishable from other febrile illnesses, particularly in children. However, when epidemic disease occurs, the characteristic triad of fever, rash, and rheumatic manifestations are unmistakable and even allow a reasonable retrospective diagnosis to be made after the passage of decades or centuries (42). MAY virus may cause disease virtually indistinguishable from CHIK. RRV infection (epidemic polyarthritis) has similar articular manifestations, but fever and constitutional symptoms are less. In those regions where SIN infection occurs, the clinical picture may also be similar, although in one SIN epidemic characteristic vesicular palmar lesions were present. Rubella, the prodrome of hepatitis B, parvovirus infection, and a variety of rheumatic diseases are all capable of causing clinical confusion. Most arboviral infections present with fever,

myalgia, and sometimes rash, but rarely with true arthritis or prominent arthralgia (329).

Virus isolation is readily accomplished by i.c. inoculation of suckling mice, although mosquito inoculation (301), mosquito cell culture, or mammalian cell culture may be equivalent or preferable for some strains (215,396). Viremia will be present in most patients during the first 48 hr of disease and may be detected as late as day 4 in some patients (40,41,142,163, 313,370).

The high viremias in these patients are also associated with antigenemia of such magnitude that hemagglutinin was detected in two of five sera tested (viremia 7.3 and 7.5 log SMICLD$_{50}$ and HA titer 512 and 1,024) (41). Thus, it is not surprising that rapid antigen detection by ELISA is feasible in about half of viremic human sera (428).

HI antibodies appear with the cessation of viremia, sometimes briefly coexisting with infectious virus. Virtually all patients will be HI positive by day 5–7 of illness (41,182,260,313). CF antibodies rise somewhat more slowly, but virtually all patients are positive by the third week; they persist in decreasing titers at least 9–12 months.

Neutralizing antibodies (mouse protection) parallel HI antibodies in the limited number of tests performed. No studies of acute sera using sensitive plaque reduction tests are available.

Virus-specific IgM antibodies are readily detected by capture ELISA in patients recovering from CHIK infection (428) and they may persist in excess of 6 months (266). In one study (266) persistence of joint symptoms did not correlate with persistence of IgM antibodies. IgM tests of this type cross-react with other alphaviruses of the serological complex (Table 2) so that reactivity of CHIK patients' sera with ONN, MAY, RRV, and SFV is found (38) and reciprocal reactions are established or expected. Use of the IgG ELISA in serosurveys is limited due to cross-reactions (308).

Treatment

Supportive care with rest is indicated during acute joint symptoms. Movement and mild exercise tend to improve stiffness and morning arthralgia, but heavy exercise may exacerbate rheumatic symptoms. In unresolved arthritis refractory to nonsteroidal anti-inflammatory drugs, chloroquine phosphate (250 mg/day) has given promising results (30).

Epidemiology

Africa

CHIK virus is transmitted in the savannahs and forests of tropical Africa by Aedes mosquitoes that belong

to the subgenera *Stegomyia (Ae. africanus, Ae. luteocephalus, Ae. opok)* and *Diceromyia (Ae. furcifer, Ae. taylori, Ae. cordellieri)*. The vertebrate portion of the cycle is provided by nonhuman primates such as *Cercopithecus* monkeys or baboons, which amplify and maintain virus circulation. It is thought that endemic circulation and moving epidemics in troops of primates are responsible for survival of the virus and local spillover into the human population. This concept is supported by field observations on mosquitoes and monkeys (27,194,276,282,283,285,295), as well as laboratory findings of high viremia in monkeys (279) and favorable characteristics of the mosquitoes for transmission (reviewed in ref. 215). The determinants of virus circulation are probably the immune status of local monkeys and climatic factors (rainfall, temperature) regulating vector density and competence. Transmission occurs primarily in rainy season when mosquito densities are highest and mosquito infection rates may exceed 3/1,000. Human involvement is largely secondary and may take the form of scattered cases presenting to clinics (275), more extensive but still localized epidemics (278,285), or brisk miniepidemics among nonimmune groups visiting areas of intense virus activity (120,278).

Tree-dwelling prosimians (bushbabies, *Galago senegalensis*) and certain species of bats have been found infected in nature and develop substantial viremias (27,215); but if they have a role in virus maintenance, it is likely to be of secondary importance. At least one rodent, *Mystromys albicaudatus*, develops a viremia potentially infective to mosquitoes (281), but field evidence does not suggest that it or other rodents play an important role.

In African villages or rural areas these mosquitoes may then infect humans, and the substantial viremias measured in humans (41,279) suggest that humans, in the appropriate setting, may contribute to mosquito infection leading to further virus amplification. This becomes particularly important when domestic-breeding *Ae. aegypti* are present in large numbers, a situation which may lead to village and large urban epidemics in Africa (254,298,299,438). The prototype CHIK epidemic which occurred in Tanzania in 1952–1953 resulted when *Ae. aegypti*-borne disease smouldered through multiple villages over an expanse exceeding 5,000 km² (254). Ibadan, Nigeria, provides an example of a city which may suffer such CHIK epidemics recurring at intervals when susceptibles accumulate (438).

Although the bulk of CHIK transmission to humans undoubtedly is mediated by the *Aedes* species discussed above, *Mansonia africana* has also been implicated as a secondary vector. It is a competent vector in the laboratory (212,281) and feeds well on primates in the canopy as well as humans on the ground. Field

virus isolations from *Mansonia* species have often occurred in connection with simultaneous isolates from *Aedes* species, including *Ae. aegypti* (20,298), as well as in circumstances in which *Mansonia* mosquitoes were the sole vector detected (194,275,283).

Primate cycles, as well as infection of mosquitoes by humans, maintain infection rates as measured by antibody surveys from 20 to > 90% in most areas of Africa, including Zimbabwe (427), the Congo (322), Burundi (356), Angola (114,235), Gabon (369), Guinea Bissau (334), Kenya (143), Uganda (275), Nigeria (157), Senegal (354), Central Africa (368), and Botswana (236). Arid regions of Central Africa (194) and Kenya (355) or Botswana (236) and temperate zones south of 18° latitude in South Africa (215) have little evidence of virus activity.

Asia

Transmission in Asia follows a very different pattern from that seen in Africa, being primarily transmitted from human-to-human by *Ae. aegypti*. Although Asian monkeys develop significant viremia after CHIK inoculation and have been found to harbor antibodies to CHIK, they have never been shown to participate in any important way in the maintenance or amplification of virus on that continent. CHIK activity in Asia has been documented since its isolation in Bangkok, Thailand, in 1958 (167), a finding soon confirmed elsewhere in Southeast Asia, including Cambodia (60), Vietnam (444), and Burma (224). A series of epidemics, usually lasting a single year, have been reported from Sri Lanka (182), Calcutta (396), and such southern Indian sites as Madras, Vellore, Nagpur, and Maharastra State (305,323). Antibody surveys indicate that CHIK has also been active further east in the Pacific, including Indonesia and the Philippines, but apparently being replaced by RRV east of Weber's line (433). In 1983–1984, there was a significant CHIK epidemic in Indonesia after years of quiescence (407). Extensive CHIK transmission occurred in the Philippines in 1967–1968 (40,259) and recurred in 1985–1987 (50,266).

The most detailed information is available for Bangkok, Thailand, over several years and for the 1964 epidemic in southern India. The discussion of these two situations will help the reader understand general patterns of CHIK epidemiology in Asia, which appear to be similar but are less well documented. The first CHIK virus isolations in Thailand were from Bangkok in 1958 in a setting of intense DEN virus activity and DEN hemorrhagic fever (167). Judging from antibody surveys (Fig. 1), the virus continued to be transmitted until 1962–1964, when detailed studies were undertaken (163–165,313). During this period, human infec-

tions occurred at formidable rates in the Bangkok area and its environs. Six percent of febrile outpatient visits and 8% of hospitalized patients suspected of hemorrhagic fever were suffering acute CHIK virus infections. In 1962 an estimated 40,000 patients sought medical attention in this urban complex of 2 million inhabitants, and 31% of a prospectively studied cohort seroconverted to CHIK virus. This intensive transmission was accomplished by large populations of *Ae. aegypti* breeding in water storage jars ubiquitous in Thai homes as a consequence of the lack of a piped water distribution system. These mosquitoes, biting voraciously indoors, had infection rates of 0.8–1.4/ 1000. The lack of breeding sites within houses was presumably the major factor that minimized infections of expatriates, whose seroconversion rates were 0.5% per year or less in prospective studies. Similar conditions obtained through the mid-1970s before CHIK transmission virtually disappeared. CHIK antibodies were rare in Bangkok children born after 1976 and virus isolations were not obtained from febrile outpatients and hemorrhagic fever suspects tested in 1979–1980 (36). The reasons for the decline in CHIK transmission are unclear, since *Ae. aegypti* were abundant and DEN transmission continued. In 1988 evidence of CHIK transmission in Thailand was obtained once again and may represent a resurgence of CHIK as a major human disease problem there.

The epidemic occurring in southern India (Vellore, Madras, Pondicherry) in 1964 provided a glimpse of another pattern of CHIK transmission in Asia, which was particularly well documented because Vellore was the site of ongoing DEN studies (41,85,305,347). Retrospective serological work showed that CHIK had not been active in the Vellore and Madras areas for about 30 years, although Calcutta had experienced epidemic transmission the previous year (396). As rainy season progressed into July, August, and September 1964, *Ae. aegypti* populations rose to a peak (Fig. 3). Human CHIK cases had first been detected at a low level in

August and rapidly accelerated through September, reaching their peak in October when *Ae. aegypti* numbers were falling. By the end of October, only occasional human cases were seen. Infection rates in *Ae. aegypti* closely paralleled human disease: 17/1,000 in September, 12/1,000 in October, 5/1,000 in November, and 1/1,000 in December. Numbers of *Ae. aegypti* decreased further with cool temperatures and drier weather. This same transmission season had been seen with DEN in previous years. Febrile illness, usually accompanied by characteristic joint pains, varied from 8 to 86% in different neighborhoods and correlated with *Ae. aegypti* densities. Males and females were equally affected, but clinical attack rates were lower in those less than 1 year of age.

It is difficult to accurately assess the impact on Vellore, but it was substantial. There were 288 laboratory-confirmed CHIK infections from whom 233 virus isolations were made, including 1 infant that died. The hospital student health clinic noted a rise in bed occupancy from the usual 10–15 to 40–50, while absenteeism among staff was 30–40% during the epidemic. Clinical disease rates in city-wide surveys averaged 44%. One small serosurvey, after the epidemic, found 15% antibody rates in those under 30 years of age and 28% in older persons.

A survey in nearby Madras City (population 1.8 million), based solely on clinical disease, revealed considerable neighborhood variation in the date of onset of epidemic disease and the eventual extent of involvement. Overall attack rates were 21% with male and female equal, but with significant age differences: <1 yr = 9%, 1–29 yr = 22%, and ≥30 years = 14%, possibly reflecting protection from previous exposure in older age groups (397).

Other Epidemiological Considerations

Although there are occasional isolations from mosquitoes other than *Aedes* and *Mansonia*, as well as
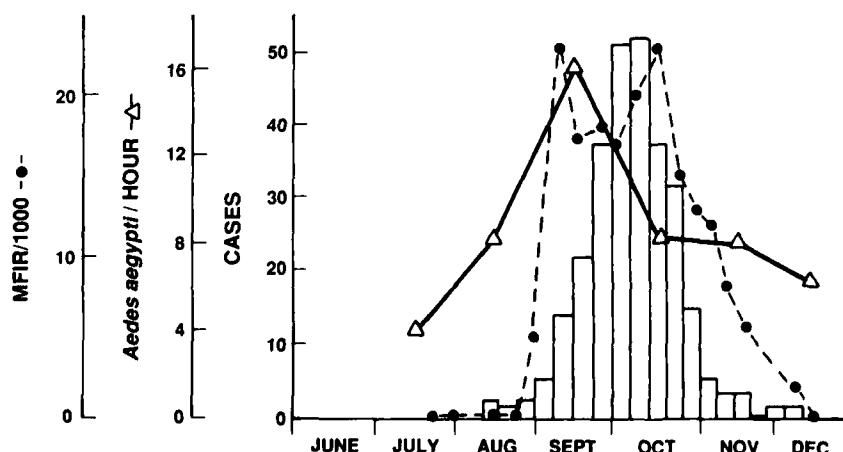

FIG. 3. Chikungunya virus epidemic in Vellore, South India, 1964. Laboratory-confirmed CHIK cases are shown with *open bars*, *Aedes aegypti* collected per hour under standardized conditions with *triangles*, and the minimal field infection rate (MFIR) of *Ae. aegypti* with *closed circles*. Adapted from Carey et al. (41) and Myers et al. (305).

from ticks, and perhaps even bedbugs (364), these arthropods have no recognized role in virus dissemination.

The very explosive nature of urban CHIK epidemics has led to speculation that biological transmission by Ae. aegypti, with an extrinsic incubation period of several days, might not be able to accomplish the high infection rates which occur in humans over a period of only a few weeks. Interrupted feeding by Ae. aegypti could increase the number of hosts exposed to competent vectors and lead to mechanical transmission by uninfected mosquitoes. Field observations on day-biting Ae. aegypti in Madras suggested this possibility and led to laboratory studies with infected suckling mice (339). Colonized Ae. aegypti, initially fed on suckling mice with a viremia of $10^{9.3}$ SMICLD$_{50}$/ml, were disturbed and immediately allowed to resume feeding on a second mouse; over half of the mice furnishing the last part of the repast became infected. Infection rates were 25%, even when the termination of the blood meal occurred $\frac{1}{2}$–4 hr after interruption.

Another interesting feature of CHIK epidemiology was described from Tanzania (254). In studies of individual dwellings, there was a highly significant trend for multiple cases to occur in a hut once a single case occurred. This, of course, could be a reflection of the flight range of the Ae. aegypti vector and human habits but is also a phenomenon that could occur as a result of mechanical transmission or interrupted feeding.

The potential for CHIK transmission outside currently known areas exists. Aedes aegypti control programs have lapsed for financial reasons, and resurgent DEN in the Caribbean, Mexico, and South and Central America testify to the intensity of reinfestation with this mosquito. Of equal importance is the broad geographic area, including the southern United States, now colonized by Ae. albopictus (173). Although this mosquito has never been implicated in the field as a CHIK vector, its potential in the laboratory is thoroughly established (267,405,434), and it remains a threat for transmission and maintenance of CHIK virus in tropical and temperate climates.

CHIK virus also is infectious for humans and animals by aerosol. This plays no role in the natural history of the disease but has resulted in a number of laboratory infections and requires that laboratory results from inoculated animals be interpreted with caution unless stringent means to prevent cross-contamination have been employed.

Comparison to Flaviviruses

The flaviviruses yellow fever (YF) and DEN 1–4 provide instructive contrasts that highlight some features of CHIK epidemiology. YF, like CHIK virus, is maintained in African forest and savannah regions in a primate cycle. Like CHIK, its transmission is thought to be regulated by the immune status of monkeys and vector density, although the mechanisms are not well understood; indeed, CHIK and YF share *Stegomyia* vectors. Transmission in these cycles leads to spillover into human populations and transmission by other vectors, particularly Ae. aegypti, which may vector village and urban epidemics. Both viruses originated in Africa and have reached (YF) or are thought (CHIK) to have reached the New World and produced epidemics. While there is no historical suggestion of CHIK transmission in the Americas after the 19th century, YF persists in a forest cycle with *Haemagogus* mosquitoes and has caused urban Ae. aegypti-borne epidemics as late as 1905 in New Orleans. In contrast, YF has never established itself in Asia, but CHIK has caused numerous epidemics and is widely transmitted there. In the Asian situation, traveling Ae. aegypti-borne epidemics appear to move through dense human populations, much as forest YF or CHIK transmission among African monkey populations.

Similarities of the DEN viruses to CHIK are also remarkable. Both cause major urban Ae. aegypti-transmitted epidemics. In the New World, CHIK apparently disappeared in the 19th century, but DEN has persisted and even increased as a disease problem with recrudescence of Ae. aegypti populations in the last two decades. In Asia both viruses are major public health problems, but their patterns of transmission differ. CHIK appears in intermittent epidemics, usually lasting a single season in the Indian subcontinent, and apparently in epidemic cycles spanning several years in areas further east, such as Thailand; DEN tends to occur in endemic or hyperendemic patterns once established. When CHIK and DEN are transmitted simultaneously by Ae. aegypti, human infection by both viruses is more frequent than expected from infection rates by either virus alone, whether evaluated by seroconversion in populations, infection resulting in milder disease, or in hemorrhagic fever patients (41,165,313). Indeed, both CHIK and DEN 2 viruses have been isolated simultaneously from the same patient (306).

Prevention and Control

Endemic disease transmission in Africa by *Aedes (Stegomyia and Diceromyia)* mosquitoes represents an unpredictable risk to humans, since many of the mosquitoes are infected from undetected monkey epizootics in the savannah or forest canopy. When virus circulates in this fashion, small groups of humans suffer high attack rates (120,278,285). The only approach likely to be helpful is the use of effective mosquito

repellents, such as deet on the skin and/or permethrin-impregnated clothing (251,385). Most recognized African epidemics, spread over broad expanses involving multiple villages or intensely attacking urban centers, have been vectored by *Ae. aegypti*. The lack of proper potable water piped to individual homes and specific local customs (254,331) make it virtually impossible to eliminate the water jars in houses and other breeding sites of this mosquito species, but addition of larvicides to the water or other innovative approaches may be possible.

Even in relatively favorable urban conditions, ultra-low volume spraying has failed to reach resting sites of *Ae. aegypti* and to provide control, even when mosquitoes are sensitive to the insecticide (121,344). Thus, control of CHIK through control of *Ae. aegypti* ultimately will require changes in economic status or cultural patterns, which do not seem likely in the predictable future. The same considerations apply to urban CHIK transmission in Asia, with the caveat that the potential role of *Ae. albopictus* and other alternate vectors has not been adequately assessed.

Prototype inactivated vaccines have been prepared and found to induce neutralizing antibodies in experimental animals and humans but have never been developed beyond initial safety studies and use in laboratory workers (170). Recently, a live-attenuated vaccine candidate has been shown to produce small plaques in cell culture, to be avirulent for suckling mice, and to produce minimal viremia in rhesus macaques (250). The neutralizing antibody response and protection in monkeys were excellent, and there was no evidence for reversion on serial passage. This vaccine has now been tested in 20 human volunteers, with no significant reactogenicity and a good neutralizing antibody response (265). Viremias were low and initial results suggest infection of mosquitoes is virtually impossible. If this vaccine candidate continues to fulfill its initial promise, further testing will be justified. The high titer of the stock vaccine (10^9) could result in a very economical product when diluted to final use infectivity (10^5 PFU/ml).

O'nyong-nyong Virus

This virus is considered antigenically as a subtype of CHIK virus; although similar in many other respects as well, there are highly significant differences.

ONN virus first appeared in February 1959 as the causative agent of a major east African epidemic (158,403). The affliction was referred to by several local appellations with similar meanings, but ONN was chosen from the Acholi word meaning "weakening of the joints." From its initial appearance in epidemic form in the Acholi Province of northwestern Uganda, the virus spread southeasterly through Kenya and Tanzania, reaching Malawi and Mozambique, with transmission ceasing in the mid- to late 1960s. The epidemic involved at least 2 million people; clinical and serological attack rates varied in villages but often exceeded 50–60% (461). Once virus transmission was detected in a village, it might last several months, although the bulk of disease usually occurred during a 2–4-month period. Clinical attack rates ranged from 9 to 78% and were somewhat less than the serological rates (403,461). The relentless progression of the disease through Uganda proceeded at an average pace of 3 km/day. Antibodies preferentially reactive with ONN have also been reported from Central Africa (62), suggesting the possibility of spread through central African savannah to the west.

After the epidemic, ONN was not heard from again until its isolation from *Anopheles funestus* mosquitoes in the Kano Plain, Kenya, in 1978 (205). Retrospective analysis of serum antibodies in that area suggested that ONN or a related agent had been transmitted with some frequency, although antibody rates were nil in 1–2-year-old children bled in 1975 (269). These data shed no light on the origin of ONN virus, which may have existed unrecognized before the 1960s epidemic or may have emerged as a mutant or recombinant virus (see EEE–SIN–WEE data in Genetics section). Today ONN may well exist in savannah and forest regions of tropical Africa but is only transmitted at appreciable rates when circumstances are ripe, reflecting favorable densities of susceptible humans and competent vectors.

The vectors of ONN are *An. funestus* and *An. gambiae*. *Anopheles funestus* appeared to be more important in the east African setting since it was more prevalent in human-biting collections, virus isolation rates were higher, and its distribution correlated better with disease incidence (71,460). Virus isolation rates of 7.8–8.0/1,000 for *An. funestus* and 1.2–4.3 for *An. gambiae* have been obtained in east Africa. Although circumstantial evidence suggests that several Bunyaviridae as well as other viruses are transmitted by anopheline mosquitoes (329), the ONN epidemic is the only major arbovirus epidemic ever recorded vectored by mosquitoes of the genus *Anopheles*. The habits of *An. funestus* resting inside huts and biting in the evening presumably were responsible for the focal nature of ONN infection observed within villages and for the clustering of cases within a given house or compound (403). The nonhuman vertebrate reservoir of ONN, if one exists, is unknown.

The pathogenesis in humans is also unknown. Viremia is regularly detected during the initial 3 days of illness and one isolation was made as late as day 6. The only other vertebrate that develops disease after ONN inoculation is the intracerebrally injected infant

mouse. Field isolates produce runting, alopecia, and occasionally a rash, with adaptation to uniform virulence after serial passage (446,458). Vervet and red-tailed monkeys, implicated in maintaining CHIK virus, developed neither disease nor antibody after ONN inoculation.

ONN and CHIK viral infections result in similar clinical syndromes (403). After an incubation period thought to be at least 8 days, the sudden onset of joint manifestations heralded the disease. Rash occurred in 60-70% of patients an average of 4 days later, often accompanied by an improvement in symptoms. The morbilliform eruption lasted 4-7 days before fading. Fever was less prominent than in CHIK infections, exceeding 101°F in only about one-third of outpatients. In contrast to CHIK, lymph nodes were markedly enlarged and of a firm, rubbery consistency. All recognized patients recovered with symptomatic therapy. Correlation of surveys for clinical disease with sero-surveys suggests that most infections produced typical symptoms (458,461).

Diagnosis has been achieved by isolation of virus in mice; serial passage or subinoculation into chick embryo cell culture is usually necessary. As with CHIK virus, diluted serum has yielded isolates where undiluted material failed (458). One strain has been isolated directly in chick embryo cell culture (459). Although never tested with field material, ONN may well be one of the alphaviruses for which mosquito cell culture or mosquito inoculation would be particularly effective.

Once isolated, viral identification can be made by cross-neutralization, HI, or CF tests comparing the strain to prototype viruses using single-injection CHIK and ONN mouse antisera; ONN or CHIK antisera react equally well with ONN virus, but CHIK virus scarcely reacts with ONN antisera. Multiple-injection mouse antisera or rabbit antisera to ONN may react extensively with CHIK virus. This is clearly a flimsy serological difference, but the behavior of ONN in infant mice and humans differs significantly from that of CHIK, and the vector relationship of ONN is quite distinct.

Patients seroconvert by HI, neutralization, and CF assays. Cross-reactions with CHIK pose a difficult problem in individual cases or serosurveys. In general, early convalescent sera from either infection react to higher HI or neutralization titer against the homologous virus, but there are appreciable numbers of individuals with similar or even somewhat higher titers to the heterologous agent (459,461). In serosurvey work, HI tests at a single dilution cannot distinguish CHIK and ONN infection (143), although determination of titers may allow limited inferences (62).

Igbo Ora Virus

This virus provides another example of the problems of alphavirus taxonomy. It is serologically closely re-lated to CHIK and ONN, although distinguishable (194,299). Using suckling mice, it was isolated from human sera in Central Africa in 1967 and Nigeria in 1966 and 1969 (89,299). The single patient with clinical observations presented fever, arthritis, rash, and sore throat. The next time biomedical science knowingly encountered Igbo Ora virus was in 1984 when an epidemic of fever, body pains, and rash occurred in four villages in Ivory Coast. This syndrome was referred to as the "disease that breaks your wings." A viral agent was isolated from *An. funestus* and *An. gambiae* mosquitoes and from sick humans. This virus, identified serologically as Igbo Ora, was obtained in cell culture and was not pathogenic for infant mice (195).

Mayaro Virus

Mayaro (MAY) virus is serologically closely related to SFV and biologically resembles CHIK. It was first recognized in Trinidad in 1954 when several isolates were made from sick patients (8). Over the next few years MAY was recognized as the causative agent of epidemics in Brazil and Bolivia (48,333). Human antibodies and/or seroconversions were recognized throughout the Amazon basin of Brazil and forested regions of Central and South America. Infection rates ranged from 10 to 60%, and there was usually a male excess reaching 2:1 over females.

Initial clinical reports had mentioned rash and in one case arthritis as ancillary features of a short febrile illness. However, in 1978 an epidemic in Belterra, Brazil, permitted careful evaluation of MAY infection in humans (244,332). In Belterra, 20% of the 4,000 inhabitants were infected, most with clinical disease. There was an abrupt onset with fever, chills, headache, and myalgia as well as arthralgia of wrists, ankles, toes, and other joints. After 2-5 days, defervescence occurred and a maculopapular rash resembling that of CHIK appeared. About one-fourth had true arthritis, and troublesome joint symptoms persisted up to 2 months. Leukopenia was common. AST was normal or elevated to levels under 100 U.

Diagnosis is readily achieved by virus isolation, preferably in Vero cells, or testing paired sera by conventional serology (332). MAY-specific IgM has been demonstrated in convalescent patients and may be useful diagnostically (38).

MAY has been isolated from *Haemagogus* mosquitoes on numerous occasions, with a handful of isolates from mosquitoes of other genera. It also causes high viremia in marmosets and other primates. Since these are the ecological elements of YF maintenance in South America, MAY may also be maintained in a similar cycle. Indeed, in the Belterra epidemic, direct evidence for this hypothesis was obtained (187). The analogy to CHIK and YF in Africa is striking, as well.

Additional work is required to evaluate other vectors and vertebrate hosts, particularly humans (viremias are very high) or birds (one isolation from a migrant) (333).

Without further information on the epidemiology and ecology of MAY, the only feasible approach to control is personal protection against vectors. CHIK and MAY cross-protect in rhesus monkeys, so it is possible that a CHIK vaccine could also prevent MAY.

Ross River Virus (Epidemic Polyarthritis)

Striking epidemics of rash and fever were noted in rural Australia as early as 1928 (314). Outbreaks in military units led to excellent clinical descriptions with a clear differentiation from DEN and other arboviral diseases; and the affliction was christened "epidemic polyarthritis" (160,404). The disease did not seem to be contagious between humans but appeared during heavy rains, leading to speculation that it might be vector-borne (9), a supposition that gained further credence when low-level cross-reactions with GET, an alphavirus first isolated in Malaysia, were found in sera from convalescent patients (400). The isolation of RRV from mosquitoes, and its serologic association with epidemic polyarthritis, led to further understanding of this disease (90). Both endemic and epidemic transmissions in Australia pose major public health problems. Although never fatal, the discomfort and loss of productivity from joint symptoms persist for weeks and occasionally even years. In 1979–1980, RRV became epidemic in the Pacific Islands, demonstrating its potential for epidemic spread beyond the Australian continent.

Infectious Agent

RRV is a typical alphavirus in most respects. Field strains from humans are more readily isolated in mosquitoes or mosquito cell cultures than in mammalian Vero cells, and probably more so than in suckling mice (5,324,361,435). Minor variations among RRV strains have been found with comparisons of biologic behavior in mice, distribution of plaque sizes, antigenic structure (kinetic HI, kinetic CF), and genetic structure (restriction maps of cDNA), but none of these properties has yet been related to differences of medical significance (5,106,109,137,466). Up to 5% genetic variation among strains has been deduced from restriction mapping of cDNA. The biologic behavior in suckling mice changes rapidly on passage in arthropods or suckling mice as variant viral genetic populations are selected (431).

Pathogenesis and Pathology

In infant outbred mice, the NB5092 strain induces an infection with very high viremia ($>10^7$ PFU/ml) but modest mortality. Infection sites are remarkably selective: ependyma and certain neurons in the central nervous system, perichondrium, periosteum, skin, brown fat, and muscle. Extensive growth in skeletal muscle and resulting myositis are major causes of acute morbidity and mortality. Smooth muscle is involved as well. Survivors develop aqueductal stenosis and severe hydrocephalus. The T48 RRV strain results in more aggressive skeletal muscle necrosis and uniform mortality; interestingly, it also causes myocarditis (293,304). A low dose of T48 inoculated into the footpad of 7-day-old Balb/c mice induces viremia and neurological disease (389–391). There is modest neuronal infection and necrosis, but the most important lesion is widespread focal demyelinization in cerebellum, brainstem, and cord. Neutrophils and macrophages are present by day 2 and appear to participate in myelin destruction, but the primary mechanism underlying myelin loss seems to be viral infection of oligodendrocytes. Cyclophosphamide administration suppresses serum antibody and prolongs both viremia and brain virus replication but only modestly increases mortality. Immunosuppressed animals have reduced mononuclear cell infiltrates, but the sequence of demyelination and remyelination is unchanged.

Studies of RRV infection of humans in Australia have made significant contributions. Biopsy of skin lesions has shown only mononuclear cell infiltration, perivascular edema, and, in the presence of purpura, some extravasation of erythrocytes. Viral antigen was detected in the basal epidermal layer and eccrine duct epithelial cells in both cases reported and in perivascular connective tissue of one. There was no evidence of Ig or C deposition or of vascular thrombosis (127,128).

Joint aspirates have had high proportions of lymphocytes, monocytes, and highly vacuolated macrophages. RRV antigens have been detected in joint fluid taken early after onset, but virus has never been isolated (126,128,179). Neither immune complexes nor evidence of C activation has been detected in joint fluids or serum samples from epidemic polyarthritis patients. The major histocompatibility complex appears to be involved in determining host susceptibility to arthritis, since DR7 and perhaps B12 types were more prevalent in epidemic polyarthritis patients (125,128). There was no excess of HLA B27. There was also an imbalance of Gm phenotypes, but there was no relation to serum HI titers or lymphocyte proliferative responses to RRV antigen.

The most reasonable synthesis of these data would be that RRV actually invades joints to produce the arthropathy. The virus certainly is capable of infecting

human synovial cultures with incomplete and transient CPE (73) and chronic noncytopathic infection of fused murine myoblast cultures has been reported (98). The destructive and inflammatory effects of viral CPE and/or the cellular immune response to viral antigens would then be responsible for rash and rheumatism. Recurrent joint manifestations would be a consequence of delayed eradication of RRV. Although there is no experimental basis to suggest how RRV might persist, the related togavirus, rubella, clearly does so (see Chapter 28) and regularly produces recurrent arthralgia.

One obstacle to an overall understanding of RRV pathogenesis has been the significant difference between observations of the disease in Australia and those made during the 1979–1980 epidemic in the Pacific Islands. While 7–9 days is the usually accepted incubation period for RRV disease in Australia, one patient observed in the Pacific became ill within 72 hr of arrival from a virus-free area (361). In Australia, patients usually presented to the physician with serum antibody and no detectable viremia, whereas in the Pacific epidemic, patients were antibody-negative during the first week of clinical disease (361) and virus was readily isolated, even in infant mice (1,324). Thus, although clinical disease patterns were similar, there seemed to be significant differences in the virological events observed, albeit with some difficulty inherent in comparing field data among different laboratories. Pacific virus strains were clearly RRV in antigenic analyses (109); they shared a single cDNA restriction enzyme pattern identical with one of the Australian continental types observed (106). The most likely explanation for the disparity is that the Pacific epidemic was caused by a RRV strain with a short incubation period and early joint invasion, compared with virus strains infecting previously studied Australian patients.

The lack of fever and other systemic symptoms during acute RRV infection and the low NK-cell activity measured in peripheral blood (3) suggest that the interferon response is not high.

In addition to the vigorous antibody response noted in RRV infection, strong T-cell reactivity is also induced. Interestingly, in 8 of 18 patients tested later, more than 20 weeks after disease, inactivated RRV antigen at the single concentration tested suppressed DNA synthesis in the cultures (3). Although individual patients with persistent arthropathy and high RRV IgM ELISA antibodies have been noted (44,128), there has been no report of systematic studies of IgM antibodies in relation to duration of rheumatic manifestations, and neither RRV HI nor lymphocyte stimulation correlated with disease activity (3,126).

In mice, RRV invades the placenta and causes fatal fetal infection (291). It has been suggested that RRV causes fetal infection in humans (2), but this finding awaits confirmation (7,362).

RRV has been circumstantially associated with musculoskeletal disease in horses, but experimental inoculation of a few equines has failed to confirm this (222).

Clinical Features

In Australian cases, the incubation period deduced from travel histories has been estimated as 10–11 days based on epidemic polyarthritis patients (9) or 7–9 days from serologically positive cases (124). Seven of 14 and 3 of 20 cases, respectively, had minimum incubation periods exceeding a week; and in all cases, it could have been a week or more.

Several series, consisting largely or entirely of virologically confirmed cases, provide a reasonable picture of human RRV infection in Australia (65,128,172,302,392). Onset is relatively sudden and the first symptom is usually joint pain. Rash occurs in the majority of patients, usually coincident with, or 1–2 days after, initial symptoms; but in some cases rash preceded joint pains by 11 days and followed them by as much as 15 days. The eruption is usually macular, papular, or both and occasionally is accompanied by vesiculation of the papules or petechiae. The eruption is typically most prominent on the trunk and limbs but may also involve the palms, soles, and face. In a minority of patients it is itchy, and it fades within a few days, sometimes leaving a brownish discoloration or desquamation. Constitutional symptoms such as fatigue and lethargy occur in only about half of patients. Body temperature is normal, or in half the patients modestly elevated for 1–3 days. Myalgia, headache, anorexia, and nausea are common. Photophobia, respiratory symptoms, and adenopathy occur but are not prominent.

The three-fourths of patients that have joint manifestations are incapacitated for considerable periods of time. Patients are kept from sleeping, walking, or grasping everyday objects by the severity of the pain. Involvement of multiple joints, often asymmetrical and usually migratory, occurs. Wrists, ankles, metacarpophalangeal, interphalangeal, and knee joints are most commonly involved, although toes, shoulders, and elbows are also targets. Joints of the spine, hip, and jaw are least often affected. These arthralgias are worse in the mornings or after immobilization; modest exercise may improve them. About one-third of patients will have true arthritis. Periarticular swelling and tenosynovitis are also common. Ten to 30% of patients will have paresthesias and/or pain in the palms and

soles; this is due to carpal tunnel syndrome in some cases.

Most patients will be unable to work or perform housework; but by 4 weeks, half will be able to resume normal activities, albeit with residual arthralgia. About 10% will still be limited by joint symptoms at 3 months. During this time, the arthralgia and arthritis run a relapsing course but on balance gradually improve. Occasional patients will continue to have signs and symptoms of articular disease for 1–3 years. Other than their rheumatic involvement, these patients are basically healthy and do not develop weight loss or destructive arthropathy.

The clinical laboratory is of limited diagnostic utility. The total leukocyte count may be normal or low and the differential is variable. Platelet counts are normal, as are other laboratory tests, including liver function tests. The sedimentation rate is usually elevated acutely and gradually returns to normal over several weeks, even in those patients with continued rheumatic symptoms and signs. C-reactive protein is less often elevated. Antinuclear antibodies are not found and rheumatoid factor is rarely elevated.

Joint aspiration yields a viscous opalescent fluid with 1–60 × 10⁹ cells/liter. During the first week of illness, five of six patients have had intracellular RRV antigen demonstrable in smears by FA. Macrophages and monocytes with intense cytoplasmic vacuolation are the predominant cell type with most of the remaining cells being small lymphocytes. As the disease evolves, macrophages appear less vacuolated and small lymphocytes predominate. Neutrophils make up only 1–12% of cells in any sample. C3 and C4 are not depleted in serum or synovial fluid (66,126,128,179).

Several outbreaks of epidemic polyarthritis, described before RRV was isolated, resemble the clinical picture specifically associated with RRV infection of humans today (9,160,314,404,451). There are interesting differences of unknown significance. For example, vesicular lesions were more commonly noted in the early epidemics, and the frequency of myalgia was quite variable among series. In individual case series, adenopathy might be particularly prominent (160); rash might precede joint symptoms (404); or swollen ears, such as described for one CHIK epidemic, might occur (451). These differences may not just reflect interobserver variation, since one recent retrospective questionnaire study found a 74% incidence of rash in Queensland cases versus only 43% in southern Australian subjects (302). Apparent differences seen in the Pacific are discussed under Pathology and Pathogenesis.

Although urinary abnormalities are not a feature of RRV infection, two recent reports have suggested an association between RRV and segmental sclerosing glomerulonephritis (78,129).

Diagnosis

Clinical

The typical presentation of joint pains, mild constitutional symptoms, and maculopapular rash that occurs in the majority of recognized RRV infections should pose relatively little difficulty in diagnosis for the prepared clinician given the geographical history. Individual cases of rubella can be indistinguishable, although prominent adenopathy, coryzal prodrome, or conjunctivitis would favor rubella as a diagnosis. Patients may present without rash or occasionally solely with rheumatic complaints and mimic early seronegative rheumatoid disease or systemic lupus erythematosus. Additional important differential diagnoses include parvoviral infection, hepatitis B, drug hypersensitivity, Henoch-Schönlein purpura, and other alphaviral arthritides. When the rash vesiculates, confusion with varicella may occur. Occasionally, the onset can be abrupt with high fever, stiff neck, and myalgia; in these cases, normal CSF and benign evolution differentiate RRV from enteroviral syndromes.

Laboratory

Virus has been isolated only from antibody-negative sera. Suckling mice have been used to obtain strains from arthropods, birds, and mammals (90,94). However, only one successful recovery of virus from human material, the blood of a febrile child, had been described before the 1979–1980 epidemics in the Pacific Islands resulted in multiple isolates from patients with arthralgia and other clinical manifestations. It is unknown whether these differences were related to the pathogenesis of RRV infection in mice or in humans (1,324). However, during the Pacific Island epidemic, inoculation of mosquitoes or mosquito cell cultures was shown to be quite effective in virus isolation (361,435) and these techniques were later successfully applied to Australian patients (5). Thus, the method of choice for isolation of RRV today is inoculation of acute serum into cultures of C6/36 cells with detection of viral antigen by fluorescent antibody staining of cells 48 hr later. In the Cook Islands epidemic, isolates were obtained from half the patients sampled in the first 2 days of illness and positives were obtained as late as day 7. Vero cells do not appear to be sensitive for primary isolation (435). Virus in serum is relatively stable and has survived abusive handling at 0 to −10°C for up to a month.

Antibody appears early in Australian cases; only 1 of 17 patients gleaned from the literature failed to have HI antibodies in sera drawn during the first week (65,90,91,93). CF antibodies are not generally present

in the first week of illness and may demonstrate seroconversion in patients who have high, constant HI titers. Although many early convalescent titers are lower, a single HI titer of ≥640 is suggestive of recent infection (1,22,93). There is some cross-reactivity with other known Australian arboviruses (Getah, SIN, and Bebaru), as well as CHIK, but neutralization tests can be used to increase specificity (91,218,433). In the Pacific Islands, HI antibodies appeared later in the course of disease, initially being detected after the first week of symptoms (361).

Many Australian RRV infections present to the physician for diagnosis only after the acute joint symptoms fail to resolve and there may be no history of rash; this, coupled with the rapid plateau of conventional serological titers, has led to considerable reliance on the detection of virus-specific IgM antibodies. This has been accomplished by indirect fluorescent antibody tests, sucrose gradient ultracentrifugal separation of 19S HI antibodies, ELISA using purified viral antigens, and an IgM antibody capture ELISA (44,321).

The IgM capture ELISA appears to be the method of choice (44). Most patients develop antibodies within the first 5–10 days of illness, with peak optical densities and end-point titers occurring during the first 4–8 weeks of illness. Reactivity began to decline subsequently, but this was more evident in the optical density (i.e., the specific activity of RRV IgM in total serum IgM) than the titer. Some patients will be positive as late as 1–2 years after infection (94,302). There are low-level cross-reactions between CHIK and RRV IgM ELISA that could be confusing in an individual patient tested against a single antigen (38).

Treatment

No systematic studies of anti-inflammatory agents have been performed. Aspirin and, if no relief is obtained, nonsteroidal anti-inflammatory drugs should be used for relief of joint pains. Since eventual complete recovery is virtually assured, steroids should not be used.

Epidemiology

Australia

RRV is endemic and epidemic in tropical and temperate regions of Australia (222,302). Unreported individual cases or small outbreaks occur annually in many regions when polyarthritis cases are sought (65,67,93). Queensland reports 300–600 cases annually, often clustered along the coastal region. As one proceeds to the cooler southern climates, endemic activity occurs along the New South Wales coast as far south as Nowra (65,67) at 35° S latitude. Antibody rates in normal populations in the temperate coastal zone tend to be low (6–15%), but if one proceeds west, crossing the highlands into the plains of the Murray Valley River system, seroprevalence reaches 27–39% (22).

Large epidemics have been reported from Northern Territory (160), Queensland (404), Victoria, South Australia (392), and New South Wales (172). Epidemic transmission occurs in river valleys or irrigated lands; high rainfall is always an antecedent, and local residents usually volunteer that mosquito populations are very high. The most recent substantial epidemic occurred in southeastern Australia. In towns along the Darling and Murray River systems attack rates, based on serologically confirmed clinical disease, ranged from 27 to 1,070/100,000 inhabitants and 3 of 180 (1.7%) of one cohort seroconverted (172).

In any given area a few dozen or a few hundred cases can occur, typically involving residents of small towns or suburbs of cities or people with outdoor recreational habits, although purely urban cases are reported (2,302). Endemic and epidemic transmissions may occur spring through fall.

Infection rates in several studies have ranged from 0.2 to 3.5% per year, which leads to a low ratio of reported cases per infection. For example, in Queensland, a 3% annual infection rate was estimated with a 0.025% annual disease rate (2). There is probably considerable underreporting of mild cases. During epidemics in New South Wales (172) and Fiji (1), the majority of infections have been symptomatic. Females predominate in cases, but male/female infection rates are similar. Children also have lower case/infection ratios.

The natural vector–reservoir relationships of RRV are not well established (222). The virus was first isolated from *Aedes vigilax* and both field and laboratory evidence have supported its role as a major vector along the eastern coast of Australia (90,94,137). This mosquito breeds in salt marshes (both coastal and inland) and is a major pest mosquito. Transovarial transmission of RRV by intrathoracically inoculated *Ae. vigilax* has been reported (221); this represents such an unusual mechanism for alphavirus maintenance (363) that confirmation is required. Further south in Australia, *Ae. camptorhynchus* assumes a similar ecological niche and is probably as vectorially important as *Ae. vigilax. Culex annulirostris* is an important freshwater breeding pest mosquito in Australia, is a competent RRV vector, and has yielded the largest number of RRV strains. It is probably an important summer vector of RRV. Several other Australian *Aedes* species and *Mansonia uniformis* may play roles as well. Antibody prevalence and viremia response to experimental infection have identified several mammalian species

as potential amplifiers, including marsupials, domestic animals, and rodents (94,222). Field observations have suggested the New Holland mouse and the wallaby as particularly important hosts in two separate settings. Low, transient viremias occur in birds, making them unimportant vertebrate hosts for RRV.

Pacific Islands

Prior to 1979, human antibodies to RRV had been documented in New Guinea, the Bismark Archipelago, Rossel Island, and the Solomon Islands (433). RRV appeared to be highly endemic in lowland regions of West New Guinea and Papua, New Guinea. Indeed, RRV is responsible for 6% of diagnosed arthritis patients admitted to Papua, New Guinea, hospitals (387). As one proceeds from New Guinea to the west, one crosses the hypothetical Weber's line, which demarcates the Australian biogeographic zone from the Asiatic zone, and RRV antibodies are no longer found but are replaced by CHIK antibodies. To the east of the Solomon Islands, neither RRV nor CHIK antibodies were detected.

These generalizations concerning the eastern Pacific Islands changed radically in April 1979 when several cases of epidemic polyarthritis occurred near the international airport on Viti Levu, Fiji (1). RRV spread rapidly through the Fijian Islands, resulting in 50,000 clinical cases. Peak transmission on Viti Levu lasted about 6 months and by 2 years no further cases were reported. RRV antibody prevalence rates in the wake of the epidemic often exceeded 90%. By August 1979 the disease was found in American Samoa (435) with subsequent involvement of Wallis and Futuna Islands, New Caledonia (324), and the Cook Islands (361). Spread probably resulted from air travel by viremic humans. The vector is unknown, although in the Cook Islands virus isolates were made from Ae. polynesiensis (361). This mosquito is a competent vector in the laboratory (156), abundant in the eastern Pacific Islands, and readily bites humans. Of particular importance, Ae. aegypti is present in the islands and a competent laboratory vector; it may have participated in transmission and could conceivably have the potential to vector RRV in areas far removed from the Pacific Islands. Culex annulirostris and Ae. vigilax are also present in some of the Pacific Islands and may have been involved as well. The high attack rates in humans and the high viremias measured in humans (361) strongly implicate humans as the vertebrate amplifier. Lesser antibody rates were measured in domestic animal and rodent species with lower potential for viremia. As with explosive CHIK epidemics, one might speculate on the potential role of mechanical transmission, demonstrated in the laboratory with Ae. vi-

gilax between mice (221). Another parallel with CHIK involves the sharing of mosquito hosts with certain flaviviruses; Ae. polynesiensis and Ae. aegypti are both important dengue vectors in the Pacific.

Sindbis

SIN virus was isolated from Culex mosquitoes collected in the Egyptian village of Sindbis in 1952 and is the prototype alphavirus (430). It has served as a model virus in myriads of studies of viral replication, viral biochemistry, membrane biogenesis, and murine viral encephalitis, but relatively little is known about its human disease potential. Because of its precedence, it has been a common antigen used in serosurveys, but since these were often conducted with the cross-reactive HI test, they tell us little more than that some alphavirus was present. Nevertheless, it is clear that SIN does infect humans, usually without recognized clinical disease, but in certain defined regions, and is a significant cause of fever, arthritis, and rash.

Infectious Agent

It is somewhat tautological to describe the prototype of the genus as a typical alphavirus, but that is the case. It has been isolated in European, African, Asian, and Australasian geographic regions. The RNA genomes of SIN strains have been compared by oligonucleotide mapping and hybridization, allowing differentiation into Palearctic, Ethiopian, Oriental, and Australian zoogeographic regions. Antigen analysis separates SIN strains into two major geographic subdivisions (Oriental–Australian and Palearctic–Ethiopian). Since all differences appear to sort by geography, as opposed to animal source, passage history or year of isolation, this Old World virus appears to be separated in its evolution by at least two, and possibly four, major geographic barriers (319,346).

Pathogenesis and Pathology

Relatively little is known about the pathogenesis in humans. Isolation of virus from a vesicle at a time when viremia was undetectable and SIN-neutralizing antibody was present in serum suggests direct viral invasion of skin (264). Biopsy of skin lesions showing endothelial damage, edema, lymphocytic infiltration, and hemorrhage with occasional necrotic cells (116) is consistent with a direct viral cytopathic effect on skin structures, with participation of the immune system occurring but probably not being central to the development of the lesion.

The arthritis, like the skin lesions, appears early in

the course of clinical disease and is often present before detectable serum antibody. Immune complexes can be detected in serum during the first few days of illness, reaching a peak during the second week and declining markedly by 1 month (211). Platelet binding, platelet aggregation, and conglutinin assays are positive, but C3c- and C1q-based assays are negative. The failure to find decreased serum C3 or C4 levels (radial immunodiffusion) or to correlate circulating immune complex activity with joint manifestations suggests that immune complexes play no direct pathogenetic role. The similarity of the clinical syndrome with that of rubella and RRV, the reports of late isolation of rubella from joint aspirates (see Chapter 28), and the persistent IgM levels found in RRV, SIN (312), and sometimes rubella suggest that invasion of periarticular connective tissue and/or joints may be the primary disease mechanism.

The pathology of SIN virus has been studied extensively in mice. The prototype mosquito strain inoculated into suckling mice produced extensive necrosis of skeletal muscle, at first suggesting that it might be a strain of Coxsackie virus. The presence of extensive neuronolysis and perivascular cuffing, as well as the other properties of SIN, readily resolved this ambiguity (430). A South African strain isolated from a human skin lesion also produced an inflammatory myositis and neuronal necrosis; but in addition, Malherbe and Strickland-Cholmley (264) described necrosis of dermal connective tissue, patchy necrosis of tendons and muscle insertions, myocarditis and valvular lesions, and brown fat degeneration. Intraocular inoculation does not result in direct cytopathic change but rather leads to an immune-mediated uveitis (43). The pathogenesis of different laboratory strains of SIN in the mouse is discussed in more detail in Griffin (154).

Avian infections lead to viremia but no disease, and not necessarily lasting serconversion (284), a factor of epidemiological relevance.

Clinical Features

Relatively few human infections with SIN have been carefully studied. The first indication that SIN caused human disease came from five cases in Uganda, diagnosed by virus isolation from acute blood (232). These patients had a transient febrile syndrome, one with joint symptoms. Subsequently, human cases have been reported from South Africa (116,213,264,280, 284), Zimbabwe (258), Central Africa (142), Australia (92,128,155), Malaysia (433), and northwestern Europe (33,105,256).

Disease typically begins with rash and arthralgia, and most described patients have both. Rash may precede or follow joint manifestations by 1 or 2 days.

Fever is not usually high and may even be absent. Malaise, fatigue, and headache occur commonly but are not oppressive. Anorexia, nausea, pharyngitis, and paresthesias occur in a minority of patients. Lymphadenopathy and conjunctivitis are not prominent.

The rash begins on the trunk as scattered macules and spreads to the extremities, palms, soles, and rarely the head. Usually papules develop, and they have a tendency to vesiculate, particularly on pressure points such as soles and palms. The rash fades within a week or so in the order of appearance, leaving a brownish discoloration. Sometimes vesicles occur between fingers and toes. The rash is occasionally pruritic or irritating. In some cases it may be hemorrhagic or recur in crops.

Joint involvement is usually multiple, migratory, and incapacitating for a few days, but gradually resolves. Acute disease most commonly affects the wrists and ankles, phalangeal joints, knees and elbows, and to a lesser extent the proximal articulations and axial skeleton (105,110,248,280). Periarticular involvement is common and tendonitis or peritendonitis (dorsum of hand, Achilles) also occurs. True arthritis (248) and even joint effusions (110) were alluded to in cases occurring in the Karelian ASSR. Myalgia is also common. In Scandinavia (33,105) and the Soviet Union (256), the persistence of rheumatic complaints has been a dominant feature. For example, in a Swedish series, recurrent joint complaints were evident in almost one-third of patients interviewed 2 years or more postinfection, and a few as long as 5–6 years (312). Late symptoms tend to be reported in those with more severe acute disease and tend to involve the joints most affected acutely. As with RRV, no deformity has been described. There is no correlation of persistent arthralgia with age, virus neutralizing antibody titer, or persistence of virus-specific IgM antibodies.

Reports from South Africa (116,280) have emphasized the rheumatic manifestations less. Indeed, it has been suggested that all the involvement is of tendons and periarticular tissues rather than true arthritis (280). Direct comparisons by clinicians in the two regions would be useful in resolving these apparent differences.

Diagnosis

The clinical differential diagnosis is similar to that of RRV. In South Africa, West Nile virus may have increased circulation at the same time as SIN and because of its rash, and perhaps prominent arthralgia, should be considered (280).

Virus isolation from human serum has only been achieved in the initial Ugandan outbreak (232) and in a single case from Central Africa (142). Other attempts,

even with very early antibody-negative sera, have failed; although inoculation of mosquitoes or mosquito cells has not been attempted. In one case, virus was isolated from vesicle scrapings (264).

Serodiagnosis with conventional HI and CF tests is efficient (92,105,256,280), as are FA and mixed hemadsorption (105). Antibodies initially appear during the first 7–10 days of illness, peak soon thereafter, and neutralization or HI titers persist for years, if not lifelong. IgM antibodies have been detected by ultracentrifugation (155) or IgM capture ELISA (312). The IgM ELISA is frequently positive in acute sera and has become positive in all patients tested to date. Its diagnostic utility may be limited by the finding of IgM antibodies persisting 3–4 years in most patients.

Epidemiology

SIN is distributed over vast areas of Europe, Africa, Asia, and Australia. A recurring theme is transmission among birds, in which the virus induces viremia but not disease by ornithophilic *Culex* mosquito species (311). Human infection is relatively common in the Nile valley of Egypt, where the close proximity of humans, birds, and *Cx. univittatus* and *Cx. pipiens* mosquitoes leads to 30% infection rates. In other areas, such as India, the virus is readily isolated, but spillover into humans only results in 3% antibody prevalence (395).

The most complete studies of SIN ecology come from South Africa (213,214,280,283). The virus occurs throughout the country and is transmitted in avian populations annually, probably with human infection as well. Extensive human disease occurs in years of exceptionally abundant rainfall or when flooding occurs in arid regions. Seroconverion increases among birds, and field infection rates among the principal vector mosquito, *Cx. univittatus*, rise from the usual 0.3–0.7/1,000 to 2.4–5.4/1,000. Human antibody rates, which commonly are 1–20%, may reflect a 15% infection rate during a major transmission season. Epidemics have been described in 1963, 1967, 1974, and 1984. In the latter two, hundreds to thousands of human cases occurred, although many did not seek medical attention because of the mildness of symptoms. It is noteworthy that SIN and West Nile viruses share avian–*Culex* ecological cycles; in South Africa SIN transmission often parallels that of West Nile; and in Israel, SIN antibodies are more prevalent in humans with West Nile antibodies (17).

Significant human antibody prevalence has been found in humans in Kuwait (192), Botswana (235,236), Kenya (24), Uganda (181), Guinea Bissau (334), and Nigeria (295), as well as other countries. In Senegal, antibody rates were particularly high in the lower Cas-

amance area and the flood plain of the Senegal River, both regions of irrigation attracting migratory bird populations (345).

In Europe, symptomatic disease is primarily recognized in the region between 60° and 65° north latitude extending from Sweden through Finland to the Karelian SSR, where it is known, respectively, as Okelbo disease (105), Pogosta disease (33), or Karelian fever (256). The clinical entity has only been described since the 1970s and associated with SIN more recently. The virus has been isolated from *Culiseta, Aedes*, and *Culex* mosquitoes in the endemic zone (257,309,311). This is a disease of adults with forest occupations (lumberjack) or hobbies (berry or mushroom picking). Factors responsible for its geographic and secular distributions are not well understood. For example, in Sweden in 1982 an outbreak involved 65 virologically confirmed cases occurring solely in the month of August and between 60° and 63° north latitude; there was no obvious explanatory pattern of vector, reservoir, weather, or human activity.

The prevalence of SIN antibodies in southeastern Asia, Indonesia, New Guinea, and Australia is low (22,94,433) as befits the low disease prevalence.

Mechanisms underlying the marked variations in reported disease rates are unknown and, of course, may reflect differences in human infection rates, disease recognition, and probably also virus strain variation.

Prevention and Control

With increasing use of irrigation to satisfy world crop needs, habitats that attract birds and are suitable for *Culex* breeding will become increasingly common in Asia, Africa, and Australia. This combination may well lead to increases in human infection with SIN virus. Control of mosquitoes in such a setting requires an expensive, disciplined, integrated approach (103). Vaccination is not a likely alternative. The best option for the foreseeable future is personal protection against mosquitoes.

Babanki Virus

Forty-three isolates of Babanki virus, a Sindbis-like agent, have been made from West and Central Africa, as well as four from Madagascar. *Culex, Aedes*, and *Anopheles* mosquitoes have yielded virus. The four isolates from humans were not associated with outstanding symptomatology (195). The significance of these isolations awaits further testing or additional evidence incriminating Babanki with human disease.

Barmah Forest Virus

Barmah Forest virus was isolated from mosquitoes in Australia. Although not closely related to other al-

phaviruses by serology, it is biochemically typical of the genus.

Antibodies in humans and four acute infections have been described from New South Wales (23; R.A. Hawkes, C.R. Boughton, J.M. Naim, J. Burke, *unpublished observations*). Three patients were febrile and arthralgia was a common complaint; one had a vesicular, erythematous rash. A fourth patient presented only with polyarthralgia resembling acute RRV infection. Arthritis has not been found and arthralgias have not been persistent.

ALPHAVIRUSES ASSOCIATED PRIMARILY WITH ENCEPHALITIS

Three alphaviruses have sufficient potential for neuroinvasion and neurovirulence in humans to be recognized as causes of encephalitis: EEE, WEE, and VEE (Table 3). These viruses may propagate in epidemic proportions and they may also cause encephalitis in equines. However, their disease patterns in humans and their ecological strategies differ. EEE is the most likely to invade the human CNS and is most likely to kill or permanently cripple when it does. When WEE infects humans it is much less likely to invade or seriously damage the CNS unless the host is under 1 year of age. Most infections with these viruses are thought to be subclinical or mild febrile syndromes, although the clinical manifestations of nonencephalitic disease have not been prospectively studied. VEE, however, is an important cause of prostrating febrile illness in humans and that is its dominant clinical manifestation. It is well established that VEE causes human encephalitis, but it is not a frequent occurrence and remains to be carefully quantified.

Eastern Equine Encephalitis Virus

Retrospectively, epidemics suggestive of EEE have been recognized as early as 1931, but the virus was first isolated by TenBroeck and Merrell in 1933 (175,432). It was quickly established that the virus was distinct from WEE, that its natural cycle was related to the salt-water swamp habitat, and epidemiological evidence adduced to support a mosquito cycle with avian reservoirs (432). These findings have been amply confirmed and extended. Eastern equine encephalitis is an established cause of severe and fatal human encephalitis along the Atlantic and Gulf coasts of North America, as well as a few inland foci in Ontario, New York, Michigan, and South Dakota.

Infectious Agent

EEE is a typical alphavirus which is notable for its relative high mortality rate when it causes human or equine encephalitis and the mortality it induces among pheasants and certain other avians. North American strains can readily be differentiated from most Carribean and South American strains by kinetic HI tests. Much less is known about the pathogenic potential and epidemiology of EEE outside North America (436).

Pathogenesis and Pathology

Relatively little is known about the pathogenesis in humans. Viremia occurs soon after infection and may be accompanied by a febrile prodrome. In a relatively high proportion (estimated in one study as 1 in 23) (149), virus gains access to the nervous system and results in severe encephalitis. Viremia is usually undetectable when acute sera are tested and HI or neutralizing antibody is present in samples taken during the first 3 to 5 days of encephalitis (147). In spite of this evidence of an effective humoral immune response, virus is not eradicated from the brain and neuronal destruction continues through direct cytopathic effect, inflammatory damage, and vasculitis.

TABLE 3. *Alphavirus encephalitis*

	WEE	EEE	VEE (epizootic)	VEE (enzootic)
Natural cycle	Birds–*Culex tarsalis*	Birds–*Culiseta melanura*	Unknown	Rodent–*Culex* (*Melanoconion*)
Vector to equines or humans	*Culex tarsalis*	*Aedes sollicitans, Coquilletidia perturbans*	Many	*Culex* (*Melanoconion*)
Equine amplifiers	No	Possibly	Yes	No
Human encephalitis[a]				
Encephalitis/infection	Children 1/50 Adults 1/1,000	Children 1/17 Adults 1/40	<1/100	?
Age	Any (predilection for infants)	Any (predilection for children)	Children	?
Case fatality rate	3–7%	50–75%	? 10%	?
Residua	Common only in infants	Common	Occasional	?

[a] See text. Estimates are highly approximate.

Younger children are more susceptible to EEE as judged by (a) a higher case to infection ratio, (b) more severe sequelae (112), and (c) less frequent prodrome (108). The higher case infection ratio suggests that the virus is more neuroinvasive in the immature human. Another reason for variation in clinical manifestations may be differences in the size of mosquito inocula. Using a mosquito–host system that is not field relevant, but that has heuristic value, Chamberlain and colleagues (51) showed that most Ae. aegypti inoculated a small virus dose (<100 SMICLD$_{50}$) upon feeding, but that a few injected 1,000–10,000 SMICLD$_{50}$.

The primary pathological features of EEE are confined to the central nervous system (15,108,176). Lesions are scattered throughout the cortex and are particularly severe in basal ganglia and brain stem; cerebellum and spinal cord were minimally involved. There is extensive neuronal necrosis as well as thrombosis of arterioles and venules. Inflammatory cells are widespread in lesions, perivascular areas, and meninges; the cells are predominantly polymorphonuclear in the first week, but later mononuclear cells may predominate. Virions have been visualized in oligodendrogliocytes by electron microscopy.

The pattern of EEE infection of horses is similar to that of humans (229). There is viremia for 1–3 days, asymptomatic or associated with fever. Viremia ceases and serum neutralizing antibody appears; in some horses there then ensues a severe and usually fatal febrile encephalitis. The necrotizing pathological pattern seen in humans also occurs in equines, but the prominent polymorphonuclear response is only observed in animals dying in the first 2 days of illness (228).

Clinical Manifestations

This is the most severe of the arboviral encephalitides, with a mortality of one-half to three-fourths of patients and commonly resulting in crippling sequelae (11,64,111,112,171,277,452). The typical clinical picture in adults may begin with a febrile prodrome up to 11 days before onset of disease. Regardless of the presence or absence of the prodrome, encephalitis begins abruptly, with typical manifestations being fever, dizziness, decreasing level of consciousness, and nuchal rigidity. Patients become comatose and exhibit tremors, muscular twitching, seizures, loss of abdominal reflexes, and focal signs. The majority of patients continue a relentless febrile deterioration and die within the first few days of hospitalization. Pediatric cases (108) usually have a more abrupt onset and more serious residua, with motor deficiency and serious intellectual and emotional damage in most (11). Occasional patients will have a febrile syndrome with no obvious CNS involvement, mild neurological symp-

toms, or even develop encephalitis and recover fully (64,452).

The human infection rate, even during epidemics, is low (113,146). In one study, prevalence of CF antibody in the wake of an EEE epidemic was 2.3%, with an estimated 23 milder infections for each diagnosed encephalitis case (149). Case to infection ratios were higher in the young and the old, which is in accord with the age distribution of reported cases (277,452). Although mortality in encephalitis cases under 10 years of age is not exceptional, the frequency of serious residual damage is greater.

Clinical laboratory studies typically show a leukocytosis with left shift. Spinal fluid is under normal or increased pressure with normal sugar and modestly elevated protein. Cell counts may be mildly elevated up to 2,000/mm^3 and polymorphonuclear leukocytes usually predominate, particularly during the first 72 hr.

Diagnosis

The abrupt onset of a severe febrile CNS disease is of course highly suggestive. Horse or pheasant deaths in unusually hot, wet summers and the proximity of salt marshes should raise the index of suspicion. Differential diagnosis in addition to other arboviral encephalitides would include bacterial meningitis, leptospirosis, and enteroviral infection (including bulbar poliomyelitis).

The virus can be isolated from serum during the prodrome (64), but most cases are diagnosed by testing paired sera in conventional HI or N tests (146). Very high CF titers occur in most convalescing EEE cases, but low titers have been observed in mild cases and serosurveys (64,148). IgM antibodies are readily detected in acute sera by ELISA (38). Brain histology is suggestive and virus is readily isolated at postmortem.

Prevention and Control

A formalin-inactivated vaccine is available for use in laboratory workers or others at high risk of exposure (68). This same product has been used to protect endangered whooping cranes, which are susceptible to lethal visceral infection. Similar killed products are used to protect equines, often combined with WEE and/or VEE antigens (175).

Epidemiology

EEE virus is endemic in focal habitats along the eastern coast of the United States, with a range from southern Canada to northern areas of South American including the Caribbean (297). Although most endemic foci are located from New England to Florida and

along the Gulf coast, specific inland habitats as far removed as Michigan have been described (287). Serious equine or human encephalitis is generally the first indication of an EEE outbreak. Small outbreaks of EEE occur almost annually and an average of six human cases per year were reported between 1955 and 1978. A greater number of equine cases occur sporadically, and occasional outbreaks with equine deaths numbering in the hundreds have been documented in northeastern states and Florida (297). North and South America isolates of EEE virus represent different antigenic variants of the virus and limited studies similarly suggest differences in virus transmission (46).

In North America, EEE virus is vectored by *Culiseta melanura*, which feeds almost exclusively on passerine birds. Birds serve as the amplifying host for this virus in endemic areas, and annual cycles of *Cs. melanura* transmission to both wild and sentinel bird populations can readily be demonstrated. The strict ornithophilic behavior of *Cs. melanura* restricts the likelihood of this mosquito species playing a major vector role for equines or humans; however, it obviously serves as the predominant source of virus for avian populations that can in turn provide infectious blood meals for incriminated epidemic vector species of *Aedes* and *Coquillettidia* (52,72,75,189,230). *Culex* species have been implicated as enzootic vectors for South American strains of EEE (88,95,401).

Birds exhibit an interesting differential host response to infection with EEE virus. Most wild bird populations suspected of serving as amplifying hosts for EEE virus exhibit a transient viremia of only a few days duration, followed by a vigorous immune response and the absence of any obvious signs or symptoms of disease. Pheasants are also quite susceptible to infection and suffer a high mortality upon infection with EEE virus; mechanical transmission by pecking can occur between caged birds (175). Bobwhite quail and sandhill cranes have been demonstrated to be susceptible to infection without disease, making them desirable sentinels for monitoring virus transmission to avians in endemic areas, while the virus appears to be lethal for the endangered whooping crane. In an outbreak among 39 captive whooping cranes in Maryland during 1984, EEE virus was recovered from 5 of the 7 fatalities, and EEE virus-specific antibody was demonstrated in 14 of the 32 surviving birds. Fortunately, the formalin-inactivated EEE virus vaccine prepared for human use also appears to provide protection for susceptible birds (63,83,231,462).

The restricted habitat of endemic EEE virus probably reflects the strict larval requirements for the vector, *Cs. melanura*. This species overwinters as larvae, which require a dark, organic-rich water microhabitat such as that found in association with floating root mat systems in the eastern United States (210). Population densities of the adult mosquitoes reflect a variety of climatological factors such as rainfall and temperature. Predisposing conditions for EEE virus epizootics would be expected to include large populations of *Cs. melanura* and coincident susceptible bird populations together with competent vector mosquito populations having a propensity for feeding on both viremic birds and susceptible horses and/or humans—all within the proximity of enzootic swamp foci. Infected birds are probably prerequisite for epidemic spread, since both humans and equines appear to be dead-end hosts. Such complicated requirements for amplification and spread of the virus may be responsible for the limited scope and distribution of EEE epizootics.

Epizootics and epidemics of EEE are relatively rare and geographically restricted to focal endemic areas. Outbreaks in temperate climates are generally thwarted by the onset of colder weather, with the consequent loss of vector mosquitoes and the southern migration of virus-amplifying passerine populations. Since the virus can regularly be isolated from these same endemic foci during the summer months, the search for a mechanism of virus "overwintering" or "reintroduction" has been extensive. Reintroduction of EEE virus into the northern latitudes by migrating birds appears unlikely, since infected birds circulate virus for only a few days, migrating populations exhibit high antibody rates, and South American strains of EEE virus are clearly antigenically different from North American isolates (46,75,104). Occasional virus isolations from larvae or egg rafts might suggest the possibility of transovarial or transstadial transmission in mosquito vectors as a logical overwintering hypothesis; however, extensive examination of field-collected material from endemic habitats have failed to support such a mechanism (174,377,378,449). Although a relative fragile overwintering mechanism might appear as an attractive focus for control or elimination of virus from endemic areas, major field investigations have failed to provide a clear understanding of overwintering mechanism(s) for EEE virus.

Western Equine Encephalitis Virus

The isolation of a filterable agent from the brain of a sick and dying horse that was part of a major equine encephalitis outbreak in the San Joaquin Valley of California in 1930 was a significant event for arbovirology. This isolation of WEE virus required the clandestine late night purchase of a farmer's horse from the farmer's wife in the face of an epizootic of unknown etiology that was fatal for approximately half of more than 6,000 horses involved. This event provided the impetus needed to stimulate arbovirus research in the United States for the next 20 years (290). WEE virus

was subsequently experimentally transmitted by *Aedes aegypti* to guinea pigs in 1933, and by 1938 the virus was shown to be distributed among a number of different animals and wild birds. The 1938 isolation of WEE virus from the brain of a child who died from encephalitis, followed by the 1941 isolation from naturally infected *Culex tarsalis* mosquitoes, prompted hypotheses on the ecology of the virus. Transmission by *Cx. tarsalis* mosquitoes was a credible hypothesis as early as 1943 (175).

Infectious Agent

WEE virus, as described in some of the earlier literature, is actually a WEE complex of serologically related viruses: WEE, SIN, Whataroa, Aura, HJ, and Y62-33 (55,219). Some of the earlier references to WEE virus may be confused by what is now known as HJ virus, which is regularly isolated from the eastern United States and differs from western United States isolations of WEE virus in serology, epidemiology, and presumably virulence.

WEE and HJ viruses have been molecularly analyzed and shown to be quite different by oligonucleotide mapping (440). The cloning and sequencing of the complete genome of WEE virus have resulted in the interesting hypothesis that this New World alphavirus evolved from recombinational events between another New World alphavirus, EEE virus, and an Old World virus, SIN virus (159).

Pathogenesis and Pathology

The pathogenesis of WEE in humans presumably resembles that of EEE. WEE is clearly less neuroinvasive and neurovirulent, both in humans and laboratory animals. Similar considerations apply to equines as well (175).

At autopsy (176,337,365) the significant primary findings are in the central nervous system. Multiple foci of necrosis, often without cellular infiltrate, are found in the striatum, globus pallidus, cerebral cortex, thalamus, and pons. In some areas, polymorphonuclear infiltrates occur. There is widespread perivascular cuffing and meningeal reaction. The frequent involvement of the substantia nigra is apparently not reflected in an important incidence of postencephalitic Parkinsonism (99,117).

The pathogenesis in rodent models is similar to that of other alphaviruses (154). It is of interest that WEE invaded the adult mouse heart resulting in a necrotizing myocarditis, although there are no apparent clinical correlates in humans (296).

Clinical Features

The disease usually begins suddenly with malaise, fever, and headache, often accompanied by nausea and vomiting (12,166,365,386). Vertigo, photophobia, sore throat, respiratory symptoms, abdominal pain, and myalgia are also common complaints. Over a few days the headache intensifies; drowsiness and restlessness may merge into stupor or coma in severe cases. In infants and children the onset may be more abrupt, often associated with seizures. Focal or generalized convulsions occur in almost all patients under 2 months of age, 90% of those under 1 year, and with decreasing frequency until they are only occasionally seen in adults (117). Physical examination shows nuchal rigidity, a disturbed sensorium, and a variety of pathological reflexes which vary from case to case and with time in a given case. Weakness and hyporeflexia are common; children often exhibit muscular rigidity, involuntary movements, and paralysis. After about 10 days, patients usually begin a gradual convalescence; fatal cases generally succumb during the first week. Many cases of WEE are mild and may present only as aseptic meningitis or an undifferentiated febrile syndrome.

The case/infection ratio has been estimated as 1:1150 in adults, 1:58 in children, and approaching 1:1 in infants (342). Rates of seropositivity have ranged from 7 to 34% in surveys of asymptomatic humans. LaVeck et al. (249) found a single person with a history of clinical encephalitis among 67 seropositives in Colorado. In a prospective study in Kern County, California, three seroconversions to WEE occurred for a rate of 1.2% per year, but no disease was noted (131). Infants are highly susceptible to CNS disease and about 20% of cases are under 1 year of age. For example, in a 1964 Texas outbreak there were 52 cases/100,000 population, but 809/100,000 infants, and in 1952 in California the corresponding figures were 20/100,000 and 249/100,000 (253,386). Interestingly, reported case fatality rates (usually 3–4%) are not exceptionally high in infants but rather show increased mortality in the ≥55-year age groups (253,277). Another pecularity of the WEE–host interaction is the excess of males with clinical encephalitis, averaging about twice the number of females. The sex ratio is equal in younger cases, but male predominance becomes evident by 5–9 years. This is usually attributed to an increased exposure of males due to outdoor occupations; however, serosurveys have failed to show any important male predominance in infection (131,249,340).

Five confirmed or likely cases of *in utero* infection near term have been observed (70,399). All survived an acute encephalitic illness, but three suffered severe neurologic sequelae.

During the acute illness, blood leukocytes are usu-

ally normal or modestly elevated with some left shift. CSF may be under increased pressure, but glucose is normal and protein is normal or mildly elevated. CSF cell counts may be normal or reach several hundred with a significant percentage of polymorphonuclears present.

After recovery from the acute disease, patients may require from several months to 2 years to overcome fatigability, irritability, headache, and tremulousness (99,117). Most adults will recover fully, although a few are left with motor damage, intellectual impairment, emotional lability, or seizures. Infants and children are at higher risk of permanent damage, however: 56% of infants under 1 month, 16% of infants 1–2 months, and 11% of infants 2–3 months of age had severe residua in motor and/or intellectual spheres, often requiring institutional or extensive home care permanently. Beyond 1 year of age, only occasional serious sequelae are noted. About one-third to one-fourth of children with convulsions during the acute illness will have subsequent seizure activity, sometimes beginning as long as 18–24 months later.

Diagnosis

WEE should be suspected in any case of febrile CNS disease from an endemic area. Overlap of clinical manifestations with those of enteroviral infection is common (340,386). St. Louis encephalitis (SLE) virus shares common ecological features with WEE, and cases can be differentiated only by laboratory studies, although clinical disease from SLE is more common in the elderly and infants are not usually affected.

Viremia is not commonly detectable in acute cases and diagnosis is most commonly made by conventional HI, CF, or neutralization tests. IgM antibodies are readily detected in acute sera (38). Isolation from brain biopsy or postmortem brain tissue is usually successful except in patients dying of late complications. Isolations from CSF and throat swab have been reported (365). The most sensitive isolation system for field material is probably suckling mice or embryonated eggs.

Epidemiology

The distribution of WEE virus actually includes the geographic ranges of six closely related but serologically distinct viruses: WEE, Y62-33, Highlands J (HJ), Fort Morgan, SIN, and Aura viruses. Most of these viruses are distributed throughout the Americas, yet subtypes of the Old World Sindbis virus are found in the USSR, Europe, Scandinavia, and New Zealand, while Y62-33 appears restricted to the Soviet Union. A subtype of WEE, AG87-646, has been isolated in Argentina and is presumably representative of endemic strains of South America (343).

In the western United States, WEE virus is perennially maintained in a cycle that employs birds as an amplifying host and *Culex tarsalis* as the principal mosquito vector. Transmission from the endemic cycle in recent years has resulted in only a limited number of human infections; however, major epidemics of equine disease with significant spillover into the human population have occurred. Most epidemic and epizootic activity occurs from mid-June through late September, depending on a variety of climatic conditions. The most extensive WEE epidemic on record occurred in the US western plains and Canada in 1941 with over 300,000 cases of encephalitis in horses and mules and 3,336 recognized human cases. The attack rates in this epidemic ranged from 23 to 172/100,000 with a case fatality rate from 8 to 15%. Subsequent epidemics in Kern County, California, in 1952 exhibited similar attack rates of 50/100,000 in humans and 1,120/100,000 in horses (342). A declining horse population in the United States, equine vaccination programs, and improved vector control in endemic areas have served to reduce disease incidence and the economic impact of WEE epizootics in recent years. WEE remains a significant disease in countries of South America, where equines are still important draught animals. Analysis of the circumstances surrounding a rather large series of WEE epidemics has resulted in the description of environmental factors favoring major disease outbreaks. Many of these predictive criteria have been fulfilled repeatedly in recent years, yet epidemics have not reached their expected magnitude (183). Regardless of the apparent decrease in annual activity in the United States, WEE remains a significant disease with numerous equine outbreaks and associated human morbidity and mortality each year. Of 148 human cases of arboviral encephalitis reported to the Centers for Disease Control in 1987, 41 (including one fatality) were directly attributed to WEE virus (49).

In the eastern United States, WEE virus has been isolated from birds and mosquitoes in association with freshwater swamp habitats along the Atlantic coast. The intimate association of WEE virus with *Culiseta melanura*, which occurs in greatest numbers in the swamp habitat and is almost an exclusive avian feeder, suggests an ecological restriction explaining the absence of significant disease outbreaks in this area (75). In addition, eastern isolates of WEE virus are serologically distinguishable from western isolates and are more appropriately designated Highlands (HJ) virus. HJ virus is now recognized as distinct from WEE virus and exhibits reduced pathogenicity for laboratory animals and horses (37). The lack of significant human disease during equine outbreaks of WEE infection in

Argentina may also be related to the different ecological factors controlling transmission in these areas, the absence of an efficient anthropophilic vector, and/or a difference in the virulence of South American strains of the virus.

Prevention and Control

The major effort pioneered in California toward WEE, through an integrated approach to mosquito control (103), has largely been successful, although unusual weather conditions can place stress on this approach. Changing personal habits such as screening of windows, air conditioned homes, and increasing indoor activities do provide protection (134). In the case of WEE where infants are a major target and suffer the greatest residual disability, protection of this group from mosquito exposure assumes particular importance. The burden in suffering and thwarted human potential is high, and the cost to society of caring for crippled children is greatest (99).

An inactivated vaccine is available for protection of laboratory workers and other personnel at high risk but is not indicated for the general population. Inactivated vaccines are also used to protect equines (175).

Venezuelan Equine Encephalitis Virus

Following the discovery of EEE and WEE viruses as equine pathogens, Kubes and Rios (240) and Beck and Wyckoff (16) isolated a distinct viral agent from equine encephalitis epizootics in Venezuela. VEE virus became the prototype for a complex of related alphaviruses (Table 4). Viral strains belonging to the VEE IABC groups are pathogenic for horses and have

been responsible for massive epizootics with extensive human infection. Their ecological maintenance strategy between epizootics is unknown despite considerable effort on the part of the biomedical community. In contrast, other VEE strains (ID-F, II, III, IV, V, VI), referred to as "enzootic," are not known to be equine virulent and do not cause epizootics but rather have transmission cycles primarily involving rodents and *Culex* mosquitoes of the subgenus *Melanoconion*. These viruses circulate in specific habitats, where they may protectively immunize horses against epizootic strains, and are thought to cause disease in humans. One such habitat exists in the Florida Everglades with occasional human cases occurring locally (102,468).

Infectious Agent

The viruses grouped into the VEE complex are all closely related by conventional serology; members of subtypes I through VI can be distinguished by cross-testing using conventional HI or neutralization. Finer distinctions into subtypes and variants were first made by Young, Johnson, and Gauld (470) using the kinetic HI test. Subsequent molecular studies have confirmed the kinetic HI results, except that IA and IB variants are not truly distinguishable (122,358,441). These seemingly minor serological distinctions nevertheless serve as markers for fundamental differences in pathogenicity, ecological strategies, and biomedical significance. It should, however, be borne in mind that more extensive field and laboratory testing will probably reveal apparent exceptions and additional complexities. The separation of strains into epizootic VEE (IABC) and enzootic categories seems to be reflected, at least in part, by surface properties. Thus, pH optima

TABLE 4. *Members of the Venezuelan equine encephalomyelitis (VEE) virus complex*

			Origin	Virulence		
Subtype	Variant	Representative strain	Species/location	Horses	Hamsters	Guinea pigs
I VEE	A[a]	Trinidad donkey	Donkey/Trinidad	+[b]	+	+
	B[a]	MF-8	Human/Honduras	+[b]	+	+
	C	P-676	Horse/Venezuela	+[b]	+	+
	D	3880	Human/Panama	0	+	+
	E	Mena II	Human/Panama	0	+	0
	F	78V-3531	Mosquitoes/Brazil	0	?	?
II Everglades		Fe3-7c	Mosquitoes/Florida	0	+	0
III Mucambo	A	Mucambo (BeAn8)	Monkey/Brazil	0	+	0
	B	Tonate (CaAn410-D)	Bird/French Guinea	0	+	0
	C	71D-1252	Mosquitoes/Peru	0	+	0
IV Pixuna		Pixuna (BeAn356445)	Mosquitoes/Brazil	0	0	0
V Cabassou		Cabassou	Mosquitoes/French Guinea	0	+	0
VI		AG80-663	Mosquitoes/Argentina	0	?	?

[a] Originally separated into two varieties. I-A and I-B are no longer considered distinguishable from each other.
[b] These subtypes are horse pathogenic and referred to as "epizootic" strains. The remainder are "enzootic" strains.

for interaction of surface glycoprotein with gander erythrocytes, chromatographic behavior of infectivity on hydroxylapatite columns (200), plaque size in Vero cells (271), and clearance from the circulation after intravenous injection (198,201,202) all differ between the two classes of virus. The clearance of viruses after intravenous injection appears to be of fundamental importance to their ability to cause disease in hamsters, guinea pigs, and monkeys; virus remained in the circulation for considerable periods when virulent host–virus combinations were studied, whereas rapid clearance of virus by the liver signaled a benign outcome. In occasional exceptional infections, clearance was found to be slow, but virus replicated poorly in target organs and the animal recovered.

In spite of their serologic diversity, the VEE viruses are all typical alphaviruses and grow well in newborn mice, mammalian cell cultures, and insect cell cultures (220,448). Adult laboratory rodents and, more importantly, equines differ in their susceptibility (Table 4); but, in general, epizootic strains are more pathogenic for these hosts than are enzootic viruses. The genetic and antigenic structures of these viruses suggest that enzootic and epizootic characteristics are multigenic and should be stable in nature (122,358,441).

Pathogenesis and Pathology

Patterns of disease seen in monkeys, equines, and rodents (145) share certain common features and provide insight into human pathogenesis. After inoculation of an epizootic strain, virus replicates in lymphoid tissues and bone marrow, resulting in high viremia, accompanied by lymphoid necrosis and a blood lymphopenia. This insult is often fatal in guinea pigs or hamsters but rarely in humans (207). Survivors of this phase may experience invasion of other tissues, including salivary gland, brain, pancreas, and fetus (197,241). Brain involvement leads to progressive lethal encephalitis in rodents, most horses (447), and some primates (294).

Experiments with a "Trinidad donkey," IAB VEE strain, in hamsters help put the early death versus late death into some perspective. Virus grows to high titer, producing necrosis in spleen and Peyers patch areas (197), and impairs the clearance function of the reticuloendothelial system. Bacteria invade the intestinal lymphoid tissue, leading to bacteremia, endotoxemia, and death. Treatment of these animals with broad-spectrum antibiotics prevents their early death at 4 days, but they subsequently succumb to encephalitis at 8 days (152).

CNS invasion, at least in the mouse (150), seems to proceed via capillary endothelial cell infection. Horses, burros, mice, and hamsters (145,197,208) all

have acute encephalitis with neuronal necrosis, mild to moderate neutrophilic infiltrate, gliosis, and perivascular cuffing. Purkinje cells are often involved. Monkeys usually have nonfatal disease, in which case gliosis and perivascular infiltrates were commonly present.

Pancreatic involvement is seen in mice (241), hamsters (151), and some equine studies (208) when virulent IA viruses are used. No involvement was seen when the attenuated IA-derived TC-83 vaccine strain was inoculated, although there have been abnormalities of insulin release in TC-83 convalescent hamsters (341). Neither VEE nor the TC-83 vaccine appears to have any diabetogenic effect in humans. This was particularly evident in follow-up studies to the 1969 epidemic in Venezuela, which included normal glucose tolerance tests in patients who had suffered encephalitis as infants (367).

VEE is potentially pathogenic to the fetus. In rats and mice, the virus crosses the placenta and results in fetal death; in the rat, myometrium and placenta are infected (136). Virus strain-dependent fetal infection has been demonstrated in horses under controlled conditions (216). Wenger (455) reported CNS abnormalities in babies born during and after a VEE epidemic. These abnormalities occurred in offspring of asymptomatic as well as sick mothers and no serological or virological confirmation was made. Lesions consisted of massive cerebral necrosis (456).

The live-attenuated TC-83 VEE vaccine is safe in pregnant horses (448). Direct inoculation of TC-83 into fetal rhesus monkey brains has led to microcephaly, hydrocephalus, cataracts, and porencephaly (252).

In many species, in addition to equines, there is a clear difference in the pathogenicity of the epizootic (IABC) versus the enzootic viruses (Table 4). In guinea pigs, lethality is a characteristic outcome of IABC infections, but also of infections with ID, a strain not thought to be equine virulent (201,380,447). In hamsters most subtypes of enzootic VEE are lethal, but the amount of virus replication is less; bone marrow, lymph nodes, and spleen are spared; and death occurs later from viral encephalitis (197). The immune response does not appear to be the major determinant of these differences; viremias are lower in every case, including, most importantly, equines (447). Studies in hamsters suggest that growth and dissemination of enzootic virus were restricted, perhaps due to a vigorous interferon response and/or interferon sensitivity of the viruses (197,199). The role of clearance of viruses from the circulation as discussed above is also pertinent. The resistant host rapidly removes virions from the circulation by nonimmune means and they are degraded in hepatic endothelial and Kupffer cells (198).

Interestingly, all these viruses seem to be capable of causing febrile disease in humans; their full spec-

trum of pathogenicity is still unknown. In rhesus macaques IABCDE cause similar viremia and leukopenia, but only IABC recipients had fever and signs of illness, and only IAC recipients had elevated serum AST levels (295).

The earliest significant VEE immune response is antibody directed to virion surface component (203,204). This antibody appears around day 5 in hamsters and mediates viral clearance by spleen and Kupffer cells. It precedes detectable neutralizing antibody but can be measured by precipitation of infectivity with protein-A-bearing staphylococci. Epitopes mediating humoral protection in the mouse resemble those in other alphavirus systems: a dominant protective neutralizing epitope on E2, lesser protection from a poorly neutralizing E1 epitope, and the presence of a cross-reactive, nonneutralizing E1 epitope (358). Cellular immunity has been little studied; in the mouse model, cell transfer experiments suggest that T helper activity is important in protection (338).

Clinical Features

Epizootic Viruses

The manifestations of virologically documented laboratory-acquired (45,?46,408), vaccine-related (426), and field (25,270,373,374) infections are indistinguishable. After an incubation period ranging from 24 hr to as long as 4–6 days, there is the abrupt onset of fever and malaise. Most clinical cases have a temperature of 102–105°F, chills, myalgia, headache, and lethargy. Photophobia, hyperesthesia, prostration, and vomiting are common; diarrhea and sore throat less so. Occasionally, remission of fever and symptoms occurs with recrudescence the next day. Physical findings are sparse but fever, conjunctival injection, pharyngeal hyperemia, and muscular tenderness may be seen. The usual duration of acute symptoms is 2–5 days with residual asthenia for 1–2 weeks.

The majority of infections are believed to lead to disease. Martin and colleagues (270), in a prospective virologically controlled study of 13 naturally acquired infections, found six with full-blown, incapacitating VEE, two with a milder myalgic syndrome, and five (38%) who denied symptoms entirely.

Epizootic virus infection causes encephalitis in a small proportion of cases (25,28,184,262,372). There is an increased incidence of encephalitis in children, even in virgin soil epidemics where preexisting adult viral immunity cannot cloud the issue. The difficulty in estimating the encephalitis/infection ratio is discussed under Epidemiology, but in adults it must be less than 0.5%, while in children it might be as high as 4% but probably is lower. Milder cases have nausea and vom-

iting with a decreased sensorium and other features such as nuchal rigidity, ataxia, and convulsions. More severe cases may have coma and paralysis. Fatalities occasionally occur and may reach 10–15% of severe cases hospitalized during large epidemics. Only a few cases have been followed for a year or more to assess residua and, in general, recovery has been virtually complete (25,247,372).

Some cases are reported to die after a fulminant 48–72-hour course (10), perhaps representing death due to lymphoid necrosis.

Clinical laboratory studies in epizootic and enzootic infections show similar results (25,87). Total white count may be normal during the first day or 2 of illness but then is usually low normal or depressed. Virtually all patients have a marked leukopenia during the first few days of illness and many will develop a neutropenia as well. Modest thrombocytopenia may be seen. AST and LDH are usually elevated. CSF in cases of encephalitis contains up to a few hundred cells, all lymphocytes.

Enzootic Disease

Serum antibody rates show virtually 100% infection in adults in many endemic zones (208), but relatively few clinical descriptions are available. High disease/infection ratios were found in two small series (123,371). These and other patients (87) have had the typical human VEE syndrome. Asymptomatic seroconversions have also been documented (379).

A fatal case following VEE ID infection was reported from Panama (207). A 14-year-old boy developed fulminant illness with fever, shock, and delerium terminating in coma, convulsions, vascular collapse, and death after 4 days. The major necropsy finding was lymphoid depletion with only minimal cerebral perivascular cuffing confined to the caudate lobe white matter. This case seems to represent the type of infection described in guinea pigs and some hamsters where systemic, and particularly lymphoreticular, infection is fatal rather than neurological involvement (see Pathogenesis and Pathology above).

Everglades (VEE subtype II) virus is active in the appropriate ecological zones in Florida (468) and has been implicated in three recognized cases of CNS disease of modest severity (102). All occurred in older persons with preexisting hypertension or cerebrovascular disease who recovered without sequelae.

Diagnosis

VEE should be suspected in any febrile myalgic illness in a person with prior exposure within 6 days to (a) an enzootic biotope (subtropical to tropical swamp

of forest) in the Americas or (b) a zone where epizootic disease has regularly occurred (Colombia, Venezuela, Ecuador, Peru) or is known to be occurring. Although VEE is often described as "flulike," respiratory symptoms beyond pharyngitis are not common so that confusion with typical influenza A or B is unlikely. Leptospirosis and enteroviral disease also figure prominently in the differential diagnosis.

Viral diagnosis is readily achieved by inoculation of suckling mice, Vero cell cultures, or other susceptible systems. Sera taken in the first 48 hr of illness are almost always positive, with occasional isolations made as late as day 5 (28,87,246,270,373). Throat swab often yields virus as well. VEE antigens are readily detected in ELISA tests on cell culture supernatants, but this technique has not yet been applied to patients.

HI and PRN antibodies appear as viremia disappears, and paired sera are diagnostically useful. The subtype or variant specificity of the immune response can often be inferred from careful plaque reduction neutralization test against several viral strains. IgM capture ELISA using a IA antigen is positive in the second week of illness (371). Two of five patients infected with a ID variant were IgM negative by 5–9 weeks. However, 2 of 10 recipients of TC-83 vaccine (derived from a IA variant) were still positive 15–24 weeks postinoculation (360). Acute EEE and WEE sera do not react in VEE IgM tests (38).

Encephalitis cases occasionally are viremic (184) but usually have serum antibody on presentation (262). In these circumstances later-appearing CF antibodies may be helpful in demonstrating seroconversion. IgM ELISA on serum and CSF would probably be useful but has not been validated in patients.

Sick horses provide a clue to the transmission of epizootic VEE and also a source of additional diagnostic material. Virus has been isolated from blood, oral swab, nasal swab, milk, and brain. Virus may no longer be detectable in brain tissue from moribund equines, but many successful isolations have been made from sera of apparently healthy animals in the same herd.

Epidemiology

Enzootic Strains

Horses are not amplifying hosts for enzootic VEE viruses, although they may become subclinically infected in areas where these viruses are active. They are maintained in large part by *Culex* mosquitoes of the subgenus *Melanoconion* (74,135,208,402,448). These mosquitoes live in tropical and subtropical swamps and forests throughout the Americans and breed in pools or meandering streams in relation to aquatic plants. Adults remain within shaded, moist areas but some species may invade nearby homes. They feed at dawn and dusk on a wide variety of rodents, birds, and other vertebrates. Rodents are their major vertebrate partner in maintaining VEE in these habitats, providing an abundant number of readily renewed hosts which develop viremias (470) that readily infect these sensitive mosquitoes (e.g., 1,000–5,000 PFU/ml) (74). Certain birds (448), bats (394), and perhaps other swamp inhabitants may play a secondary role. Virus is maintained through dry season by low-level transmission or by persistence of long-lived infected adult vectors.

Human populations living in these areas have high antibody prevalences, reflecting the cumulative experience with the continuous transmission that occurs. Infections among soldiers entering endemic zones (123,371) and sentinel hamster studies (86) both show that at a given point in time virus activity can be intense but spatially very focal.

Epizootic Strains

These viruses (100,208,448) have all been isolated during equine epizootics and horses play a major role in their amplification. Their maintenance between epizootics is completely unknown. Molecular studies clearly show that epizootic viruses are not simple variants of known enzootic strains. As a matter of fact, studies in Colombia and Guatemala show that true enzootic foci within regions where epizootics have previously raged and faded continue to support enzootic virus circulation (381,445). In the Guatemalan situation, the local enzootic vector, *Culex (Melanoconion) taeniopus*, was refractory to oral infection with epizootic virus; and, indeed, other vectors had been important during the previous epizootic.

It has been suggested that bats, birds, or other mammalian hosts may maintain epizootic strains in restricted foci until ecological factors, accumulation of susceptibles, and high mosquito densities are favorable for introduction and/or emergence of equine epizootics. Also, while epizootic viruses do not persist as what we recognize today as "enzootic strains," it is possible that they are maintained in nature in a genetically different form and require a mutational and/or selective event to emerge with full equine epizootic potential.

Whatever the cryptic source of these epizootic strains, epizootics have struck Venezuela, Colombia, Ecuador, and Peru at intervals of 10 years or less. Favored ecological zones are tropical dry forest and tropical thorn forest, which are areas of agricultural and cattle ranching importance. These outbreaks have occurred since the 1930s and climaxed in a massive epizootic from 1969 to 1972 that extended throughout

Central America and Mexico to finally reach south Texas. Since this epizootic, no major foci of epizootic virus activity have been identified as of 1988.

Typically, epizootics are rainy season events and often are associated with unusually heavy rains. In the center of an epizootic, transmission usually continues until most horses are dead or immune. Epizootics may spread contiguously at rates of 4–5 km/day or may jump to nonadjacent areas through transfer of infected vectors or equines (100).

Many different mosquito species from several genera have been found to be infected during epizootics, and several have been implicated as likely vectors, including *Psorophora confinnis, Aedes taeniorhynchus, Mansonia dubitans, Aedes aegypti,* and others. Thus, there is no single species complex implicated as dominant, such as the *Cx. (Melanoconion)* relationship to enzootic strains (74,239,381,448). Instead, relatively high viremias have been required for oral infection and subsequent transmission of epizootic viruses by most mosquito species (239,448), emphasizing the importance of the horse as a critical amplifier. Indeed, the high prolonged equine viremia may lead to circumstances in which mechanical transmission by mosquitoes or even black flies assumes importance (188). Many other hosts that might participate in epizootic virus transmission have been suggested, but it is unlikely that pigs, cows, dogs, bats (394), and others play more than an ancillary role except in unusual circumstances.

Although human disease typically follows horse disease by 1–2 weeks, humans do have a sufficiently high viremia to infect mosquitoes. There is at least one outbreak in which circumstantial evidence suggest that human–*Ae. aegypti*–human transmission may have been a contributory mechanism (424), but humans have never sustained epidemic transmission or been implicated as generally important. Similarly, while humans excrete VEE virus in throat wash, there is no evidence of person-to-person transmission (25,408). The virus present in oropharyngeal secretions of rodents (470) and equines (208) is also of no known epidemiological significance.

Humans in Epizootics

Clinical attack rates in humans in the epizootic setting (10,28,184,262,270,372) commonly are in the 10–60% range. When serological confirmation is attempted, one-half to two-thirds are positive. Clinical and serological attack rates are higher in males, thought to reflect differential occupational and recreational exposure to epizootic mosquito vectors. Attempts to estimate the incidence of encephalitis after VEE infection of humans pose an interesting problem

in field epidemiology. Because of intercurrent disease that may simulate VEE, subclinical infection, and incomplete reporting, use of febrile disease as an estimate of total infections is unwise. The clinical diagnosis of encephalitis or aseptic meningitis may also be difficult in a dehydrated, febrile patient prostrate from VEE and suffering from severe myalgia. Diagnosis is further complicated if the infrastructure for CSF examination and laboratory tests is not available. Even with the correct clinical diagnosis there is an inevitable admixture of other arboviral and enteroviral illnesses that also peak in rainy seasons; for example, in a Texas WEE epidemic, about one-third of the cases were confirmed as WEE, one-third as enteroviral or SLE infections, and one-third as none of these (386). With these caveats, 31,966 reported clinical cases from the 1962–1964 VEE epidemic in Venezuela had 3.8% encephalitis cases and a 0.6% overall mortality rate (28,393). However, in Guatemala in 1969 no VEE encephalitis was seen in a village with an estimated 900 infections (319). In another 1969 study in Guatemala and El Salvador, an overall 20% seropositivity rate in areas encompassing several hundred thousand inhabitants yielded only 27 reported encephalitis cases and 16 deaths (184). The only epidemic in which both infections and cases were documented was in Colombia in 1967 and yielded rates of 0.4 encephalitis cases per 100 adult infections and 4 per 100 children (372). The 1971 south Texas experience yielded reported attack rates of 5–22 per 100,000 (25). Of 79 confirmed cases studied, 8% had encephalitis and the rate was 24% for those under 17 years of age. There were no fatalities and none had residua at 1 year follow-up. Retrospective serosurveys in Texas suggested higher infection rates (0.5–3% in affected towns), so the actual incidence of encephalitis may have been even lower.

Laboratory Transmission

VEE is highly infectious by the aerosol route. Numerous laboratory infections have occurred through manipulation of the virus or defined accidents (45,246,408). Laboratory workers should utilize appropriate safety measures and avail themselves of the additional protection afforded by vaccines such as TC-83.

Prevention and Control

Enzootic VEE poses a health threat to humans in the warm, moist habitats where it is transmitted. *Culex (Melanoconion)* mosquitoes bite humans avidly but are primarily crepuscular feeders and do not venture far outside their specific ecological niche. Thus, repellents, avoidance of habitats at dawn and dusk, and

siting dwellings away from the humid shaded areas where mosquitoes are active are all useful. Vaccination is not generally practical.

Epizootic VEE should be controlled by equine vaccination. This protects the veterinary target against disease and prevents amplification of VEE virus in mosquitoes feeding on viremic horses and mules. The live-attenuated human vaccine TC-83 is safe in equines and is capable of inducing immunity within 3 days of vaccination (448). This is the vaccine of choice to interdict epizootics. Formalin inactivation of TC-83 produces a killed immunogen also satisfactory for protection of equines. Inactivated vaccines made from virulent virus strains should not be employed because of the risk of residual live virus; such products are suspected of having initiated epizootics in the past.

There is a live-attenuated vaccine developed for use in humans (286). This strain, developed by passage of the Trinidad donkey strain of IAB virus in cell culture, is designated TC-83 and has been administered to several thousand volunteers. Neutralizing antibodies develop in more than 90% and long-lasting protection against laboratory infections ensues. It was also well tolerated in immunosuppressed tumor patients when used as an experimental antineoplastic therapy (437). Nevertheless, about 15% of recipients develop moderate fever and malaise. Furthermore, the vaccine is not known to be safe in pregnant women and intrauterine inoculation of rhesus monkey fetuses has resulted in developmental abnormalities (252). Preexisting alphavirus antibodies may also prevent primary immunization, and booster immunization is difficult.

To overcome some of the difficulties inherent in TC-83, an inactivated product was prepared from the attenuated virus strain (69). This vaccine is immunogenic in itself (101) and has also been highly useful in boosting waning immunity from TC-83 immunization (35). This is particularly important in laboratory workers handling enzootic strains. The highest antibody respond following TC-83 is directed against IAB viruses and neutralizing antibody against IDE or more distantly related subtypes is lower. Laboratory infections with IDE strains have been frequent as heterologous neutralizing antibody titers decline with time.

Vector control during epizootics has a limited role due to its expense, emerging mosquito resistance to insecticides, and environmental considerations (53).

OTHER ALPHAVIRUSES

The remaining alphaviruses listed in Table 2, as well as many that undoubtedly remain to be described, are of lesser medical or veterinary significance and much less is known about them. They may be of low virulence for humans or the viruses may not have been active in an area in which the infected mosquito species readily bites humans. Any search for further information on these agents should include Theiler and Downs (436) and Karabatsos (220). Four viruses from the group deserve further mention here.

Semliki Forest Virus

SFV was initially isolated from mosquitoes in Uganda in 1944 and then was used extensively as an antigen for serosurveys in Africa, Asia, and the Americas. The rugged and inexpensive HI test was employed and many reports of "SFV antibodies" followed. These are largely cross-reactive HI antibodies from other alphaviruses. The actual domain of the virus is Sub-Saharan Africa; several mosquito isolates are available to support this (220), as well as specific monotypic human antibodies at a low prevalence in several serosurveys (235,236,295,234,410,425).

The disease spectrum in humans is not well understood. The virus is widely used as a model virus in nonmicrobiological as well as microbiological laboratories, and several asymptomatic seroconversions have been observed. However, one such scientist working with a strain of SFV developed a typical arboviral encephalitis with SFV isolated from CSF and postmortem brain tissue (463).

An intriguing observation was made in the savannahs of Senegal (352). During an epidemic of equine encephalitis, diseased horses and other animals in the herd were bled; five of six tested had high-titered neutralizing antibody to SFV. A serosurvey of normal horses in different regions of the country showed 11–29% positivity by HI, with many of these also possessing neutralizing antibodies. While far from conclusive, these data, taken with the pathogenicity of other alphaviruses for equines, suggest that SFV deserves further attention as a veterinary pathogen.

Getah

GET virus was initially isolated in 1955 from mosquitoes in Malaysia and a year later the closely related Sagiyama virus was obtained from mosquitoes in Japan. Although never implicated in human disease, GET is an important veterinary pathogen and has some interesting properties.

GET is readily propagated in mosquito cell cultures, which proved the most sensitive system for virus isolation from field samples. Different strains isolated from nature, isolates in mosquito or suckling mouse systems, and plaque variants of a single strain may differ in optimum host systems, production of hemagglutinin, properties of hemagglutinin, mouse virulence, and plaque phenotype. In spite of this, oligo-

nucleotide fingerprints have confirmed a 68–96% ratio of similarity among geographically different isolates (reviewed in ref. 237).

The virus is known to be distributed in the Asian Soviet Union, Japan, Taiwan, southeast Asia, and Australia (94,220,237). Studies of epidemic polyarthritis in Australia correlated HI antibodies to GET with the presence of disease, establishing a link to alphavirus infection (400), which was eventually solidified by the isolation of the closely serologically related RRV (90,91). Serological studies of the Pacific Basin have identified only four unequivocally seropositive humans after testing thousands of sera (218,433), and human HI antibody rates in Japan are 1–9% (237).

Interest in GET increased in 1978 when race horses in Japan suffered a syndrome characterized by fever, rash, and limb edema, apparently followed by complete recovery (217). Recurrent epidemics in 1979 and 1983 have led to the use of inactivated vaccines. Nationwide horse infection rates vary, but more than one-fourth are seropositive. During epidemics, 20–70% of infected horses have clinical disease.

Although many mosquitoes are competent laboratory vectors and/or have been the source of occasional isolates, field studies suggest *Aedes vexans* is the major vector in cooler northern climes, giving way to *Culex tritaeniorhynchus* in warmer habitats (237). During equine epizootics, horses are likely to be important amplifiers because of their high prolonged viremia. Pigs are probably also important vertebrate amplifers and may suffer fetal infection and damage. The vectors and vertebrates that were host to GET prior to domestic animal introduction or in ecological zones undisturbed by humans are unknown.

The similarities of GET to RRV are striking in many respects and may provide parallel insights into the epidemiology of RRV, as well as the closest experimental model available for the fever/rash syndrome in humans.

Una

Una virus is a close serological relative of MAY, known from Trinidad, Central America, and South America. Biologically, it seems to differ in its spectrum of vectors, with most isolates being from mosquitoes of the genus *Psorophora*. Human serosurveys are difficult to interpret because of cross-reactivity with MAY, but a number of definite seropositives have been identified. Of additional interest, two isolates have been made from sick horses in Argentina (220).

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